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16. Abstract
4 small outdoor reactors, a predator infested outdoor pond, and numerous indoor systems were designed, constructed and operated to provide data describing the kinetics aspects of algal systems used as wastewater treatment schemes. The studies included determinations of: (1) the kinetics of ammonia-nitrogen as a growth limiting factor; (2) the effect of nutrients in wastewater effluents on algal growth; (3) suggested designs for both indoor and outdoor growth reactors; and (4) predator-prey relationships in outdoor algal systems. Specific growth rate data for nitrogen were quite consistent, allowing the use of the maximum specific net growth rate as an extremely reliable parameter of system operation. Parameters K_A, K_S, and S_N all directly related to the maximum specific net growth rate, were shown to be useful both in mathematical interpretation of system kinetics and in determining to what percent of capacity an algal system is operating. Other studies demonstrated that permissible dilution ratios for waste water discharges into receiving waters can be formulated empirically. Design and operational information of various systems is presented, as well as preliminary bioassay results on the microbial populations of algal ponds. (Lowry-Texas)

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KINETICS OF ALGAL SYSTEMS IN WASTE TREATMENT

Ammonia-Nitrogen As A Growth-Limiting Factor and Other Pertinent Topics

Final Report - Part II

by

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Federal Water Quality Administration
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I. INTRODUCTION

The process of accelerated eutrophication of lakes and rivers throughout the world is a problem of major proportion which few people in the field of water pollution control need be reminded of. Already, this process has reduced many once beautiful water bodies to a state of ugliness and decay by creating gross imbalances between heterotrophic and autotrophic activity. Through the introduction of vast quantities of inorganic nutrients and with the aid of sunlight energy, a continuing symbiotic cycle between algae and bacteria creates a situation where the biomass of a water body irreversibly increases. Once inorganic material (i.e., nitrogen, phosphorus, carbon, etc.) is converted to algal biomass through the photosynthetic mechanism, the organic material thus converted tends to remain in the system. Therefore, the only change is often the transformation of the material back and forth in a cyclic process from autotrophic to heterotrophic forms. The most striking example of the impact of this destructive process is the current situation in Lake Erie. Numerous articles (1, 2) describe how during the past 50 years eutrophication in Lake Erie has advanced to a relatively high level causing a significant economic burden to be placed on the communities bordering its shores. Clear Lake, California and Klamath Lake, Oregon, are other well-known examples of this problem.

Although a concerted effort is being made by many researchers to gain a fundamental understanding of the causes of eutrophication and to develop and implement preventive and corrective measures, because of the vast complexity of the problem a number of important research areas have been left relatively untouched. Historically the major emphasis on the causes of accelerated eutrophication has been directed at determining the nutritional requirements of green and blue-green algae and in trying to discover those levels of nutrients which will support no, or at the most, minimal algal blooms in water bodies. A considerable amount of research has been devoted to analyzing the effects of varying nitrogen and phosphorus concentrations on algal growth since these two elements appear to be major causal agents precipitating many algal blooms. Mackenthun (3) has summarized the important work in this area through 1965 in an annotated bibliography and has also published a selected reference list (4) outlining the research performed through 1966 on algal growth factors other than nitrogen and phosphorus. Similarly, Stewart and Rohlich (128) have reviewed the entire problem of eutrophication through 1967.

More recent reports (5-9) have brought the problem to its current level of understanding.

Concurrent with this accelerated interest into the causes of eutrophication there has been something of a revolution in the field of Sanitary Engineering with respect to the emphasis on methods of waste water treatment. Tradionally, the sanitary engineer has approached the problem of waste water treatment by looking at the most obvious objectionable characteristics of waste waters, namely, BOD and suspended solids. Standard Textbooks (10-12) describe in detail the developments in waste water treatment aimed at removing these two characteristics to acceptable levels. In recent years, however, there has been a growing concern over the removal of nutrients, particularly nitrogen and phosphorus, from waste waters, in addition to the conventional removal of BOD and suspended solids, so as to reduce the algal growth potential of receiving bodies of water. In a recent symposium in Lake Tahoe (13) on waste water reclamation, the state of the art of removing phosphorus and nitrogen was brought up to date.

The use of algae systems for removing all of the objectionable characteristics of waste water has two inherent advantages over other methods in practice and under intensive research for accomplishing these tasks. While most methods must follow a unit operations approach (i.e. separate units for removing suspended solids, BOD, nitrogen and phosphorus - see references 14 and 15) algal systems potentially have only two unit operations, namely a growth reactor and a separation unit. Since algal systems rely on a symbiotic algal and bacteria phase flourishing in the same growth reactor, all of the required tasks of removal are accomplished in one unit, at least in the sense of converting all of these objectionable constituents into algal and bacterial biomass. Since nutrients are required for algal growth, the algal growth potential of a waste water is heavily exerted in an algal reactor thus precluding any further exertion of this growth when the treated waste water is discharged to a receiving body of water.

APPLICATIONS OF CONTROLLED ALGAL SYSTEMS

The main advantage of algal culture results primarily from the simultaneous accomplishment of biomass production, oxygen production, CO_2 absorption, and nutrient utilization and removal associated with the photosynthetic reaction. Among the more important applications are protein production, waste treatment, and biogenerative systems.

Use of Algae In Waste Treatment

In waste treatment, algal processes can be used as secondary treatment systems fed by organic wastes, as tertiary treatment for removal of soluble mineral nutrients, or as combined secondary-tertiary treatment where both the reduction of BOD as well as nutrient removal are objectives of the treatment.

Photosynthetic Oxygenation of Organic Wastes. The use of an algal-bacterial symbiotic culture system as a means of photosynthetic oxygenation in the aerobic oxidation of sewage was studied by Oswald and Gotaas (16-18) at the University of California (Berkeley) in the early 1950's. Their findings led to development of the concept of "high-rate" pond systems for waste treatment. The principal objectives of the high-rate pond system concept are BOD and algal nutrient removal, and the production of algal-biomass for use as an animal feedstuff.

In this system, BOD is removed through bacterial oxidation of the organic matter. A considerable or major portion of the oxygen used by the bacteria is produced by the algae. An added benefit of this system is that a highly proteinaceous algal product is obtained when the algae is separated from the liquid portion. This protein material could then be used as a diet supplement for livestock. This possibility was explored in the University of California studies in which it was found by Hintz (19, 20) that sewage-grown algae served as an excellent source of protein for the test animals, i.e., comparable to soybean oil meal for sheep and fish meal for swine.

Nutrient Removal. The removal of algae growth-promoting substances from waste effluents by mass cultures of algae under controlled conditions appears to be an effective method of nutrient removal. Experiments have shown that after a mass culture of algae has been grown on a waste effluent and the algae thus produced have been removed from the treated water, very little additional growth of algae can be supported by the water. Removal of a given amount of algal cellular material constitutes removal of a proportionate amount of nutrient. Depending upon the species of the algae and conditions under which they were grown, dried algae contain from 6% to 11% nitrogen and from 0.5% to 2% phosphorus (21, 22).

The high content of nitrogen in algal biomass indicates that a substantial portion of the nitrogen removal from waste waters (and natural bodies of water) is by way of assimilation by the algae, i.e., conversion of soluble nitrogen into plant protein. Other minerals also can be removed. Bush for example (23) reports a reduction of approximately 40% in total soluble solids brought about by assimilation and precipitation of K^+, Ca^{++}, Mg^{++} $SO_4^=$, HCO_3^-, NO_3^-, NH_4^+, and $PO_4^{\equiv}$ following mass algal growth.

Biological stripping of algae growth-promoting substances includes the assimilative removal of micronutrients. The trace elements Mo, V, Co, Cu, Mn, Zn, and B have been demonstrated as being essential for the growth of algae (24). In fact, one or more of them may be the limiting factor for the growth of phytoplankton and blooms in natural bodies of water (25).

Similar reasoning applies to the stripping of the more "exotic" growth stimulants such as vitamins and other biologically synthesized growth factors by the mass culture of algae. Among the vitamins, vitamin B_{12}, thiamine, and biotin have been shown to be essential to algal growth (26, 27).

It is possible to combine photosynthetic oxygenation for BOD removal and nutrient removal in one operation followed by the harvest of the algal biomass. Combined secondary-tertiary treatment has the advantages of reduced costs and increased algal yields - the latter due to an enhanced supply of CO_2 resulting from the bacterial degradation of the organic matter in the wastes. Thus far, studies on algal nutrient removal mostly have been rather limited. Experiments and observations ranging from laboratory to full-scale treatment plants have been described by Parker (38), Bogan (29-31), Fitzgerald and Rohlich (32, 33), Oswald _et al_. (34), Reid (35, 36), Bush _et al_. (23), Hermann (37), Beck, Oswald and Goldman (38), Hemens and Mason (39), Humenik and Hanna (41), Shelef, _et al_. (40) and others.

Use of Algal Culture for Proteinaceous Feedstuff Production

Advantages in the use of algal systems for production of protein-rich feedstuffs as compared with conventional agriculture have resulted in focusing attention on the use of large-scale, controlled algal production systems. The possibility of using algal systems to meet at least a part of the protein shortage in developing countries such as India, Israel, Japan, Thailand and others is becoming recognized. As reported by Enebo (42) and Mitsuda (43), pilot-plant scale installations designed specifically for protein production with a surface area of 1/8 to 1/4 acre are currently in varying stages of development in Czechoslovakia and Japan.

General features common to all algal mass culture systems are: 1) a controlled reactor illuminated primarily by sunlight; 2) a rapidly growing algal culture operated as a continuous culture; 3) a suspending medium containing mineral nutrients; 4) a provision for adding CO_2 to the culture; and 5) a means for harvesting and processing the algae.

Use of Algal Systems for Life Support Systems

The use of algae grown in mass culture under carefully controlled conditions is one of the types of life support systems proposed for prolonged space travel, space laboratories, and permanent extraterrestrial bases. The maximum dimensions of an algal photosynthetic reactor designed for a life support system is determined by its gas-exchange capacity, i.e., capacity for oxygen regeneration and CO_2 absorption. In addition to serving as gas exchangers, the reactor can function simultaneously as a device for organic waste treatment, nutrient removal, and water reclamation. The organic waste treatment function depends upon the symbiotic activity of algae and bacteria. The biomass, predominantly algal, may serve as an important food source. Thus, the algal photosynthetic reactor can serve as an integrated life support system and in this respect it is unique as compared to regenerative processes which depend upon bacteria or on chemical and physical phenomena.

A detailed discussion of the complexities and technical ramifications of photosynthetic life support systems is beyond the scope of this presentation. Excellent reviews of the state of the art is contained in an article by Miller and Ward (44). In brief, the major factors to be considered in arriving at a design for an algal photosynthetic reactor for a life support system are similar to those of an algal system designed for waste treatment. In both, light intensity, nutrient and organic loading, and CO_2 supply are major factors, and the capacities of both are determined by the net production of the algal biomass through the photosynthetic process. Apparently the major difference between the two is that life support systems are of necessity more mechanized, and are more closely controlled as far as environmental conditions are concerned. This is in order to attain a maximum degree of efficiency, and a reduction insofar as is possible in volume, weight, and surface area requirements.

OBJECTIVES OF THIS REPORT

As with the other reports in this series (45, 46), the studies within this report are concerned with describing the kinetic aspects of algal systems used as wastewater treatment devices. They include 1) the kinetics of ammonia-nitrogen as a growth-limiting factor; 2) the effect of nutrients in waste water effluents on algal growth; 3) suggested designs for both indoor and outdoor algal growth reactors; and 4) predator-prey relationships in outdoor algal systems.

Together with the first two reports, which covered light, nitrate-nitrogen and phosphorus as algal growth limiting factors, this report covers those aspects of algal reactors which require extensive understanding before high-rate ponds will take their place in the mainstream of waste water treatment technology. Although the work covered in this report deals primarily with indoor experiments on growth-limiting factors, a limited amount of outdoor experimentation was performed. Four small outdoor reactors were constructed and preliminary experiments on nitrogen removal were performed. Also, these outdoor systems were used for comparative purposes with a predator infested outdoor pond. Further experiments are now being carried out on these outdoor systems at the Sanitary Engineering Research Laboratory of the University of California at Berkeley.

The work contained within the three reports, comprising the total efforts of this project, covered a three-year span (June 1967-August 1970). Obviously this material should not be considered as the final word on algal systems for waste water treatment. Rather, it should be viewed as just the beginning of a new and important contribution to the field of waste water treatment systems development.

II. ALGAL GROWTH KINETICS WITH RESPECT TO AMMONIA-NITROGEN

INTRODUCTION

In the first progress report of this project (45) the kinetics of algal growth with respect to nitrate-nitrogen was extensively investigated. Nitrate-nitrogen, the most oxidized form of nitrogen, is found as the principal nitrogen source in many natural waters. It is this form that is very often the main nitrogen source utilized by planktonic algae found in these waters. To a lesser degree the reduced nitrogen forms such as ammonia and dissolved organic nitrogen are also found in natural waters. However, ammonia nitrogen constitutes a major source of nitrogen for algal growth in algal waste treatment systems such as stabilization ponds and high-rate ponds. It is a relatively stable intermediary in the nitrogen cycle of both natural waters and waste treatment systems since the transformations of organic nitrogen and urea to ammonia by the process of ammonification are very rapid (47) while the nitrification process, as evidenced by numerous ultimate BOD studies, is quite slow.

An understanding of the relationship between algal growth and ammonia concentration in the aqueous phase is thus important for a more complete interpretation of the growth kinetics of algae in waste treatment systems and in those natural waters which receive partially treated waste waters high in ammonia nitrogen.

LITERATURE REVIEW

An extensive literature review on the relationship between algae and nitrogen pertinent to the kinetics of algae growth with respect to nitrogen is given in Reference (45), Chapter IV. Only some of the topics related specifically to ammonia nitrogen are given herein.

With the known exceptions of *Euglena gracilis*, var. bacillaris and and *Chlamvelomonas reinhardii*, which cannot use nitrate (48), most of the algal species can utilize nitrate and ammonia, and apparently the nitrogen content of cells of algae growing in medium containing either of the two sources remains the same. Thus, Cramer and Myers (48) found the nitrogen content of *Chlorella* to be the same regardless of whether NH_4 or NO_3 was used as the nitrogen source, and the empirical composition of the cells in either case to be $C_{5.7}\,H_{9.8}\,O_{2.3}N$. It is of interest that *Euglena* and *Chlamvelomonas* often are the predominant algae in waste ponds heavily loaded with organic matter and in which anaerobic conditions often exist and the nitrogen is exclusively in a reduced form.

For a long time it was generally accepted that, as is the case for most of the higher plants (49), nitrate constitutes the optimal form of nitrogen source for algae. However, a few researchers in their studies on nitrogen-starved algae observed that ammonia was taken up and assimilated more rapidly than was nitrate. For example, Witt (50) found that approximately 80% of the NH_3-N in the substrate was assimilated by the algae before any uptake of NO_3-N occurred. The preferential assimilation of NH_4-N, if such be the case, may be due to the fact that the energy requirements involved in the utilization of NH_4-N are lower than those for NO_3-N, which must be reduced before it can be converted into cellular material.

Differences between growth on NH_4-N and NO_3-N may be a function of change in pH level brought about by the uptake of the nitrogen compound. Thus, Syrett in his review (51) states that the maximum rates of growth of several commonly investigated algae grown on NO_3-N and NH_4-N were identical, provided that interference brought about by fluctuations in pH level were eliminated. Gerloff and Skogg (52) attribute their difficulties in growing *Microcystis aeruginosa* on ammonia as compared to their absence when nitrate was used, to the drop in pH level that accompanied assimilation of the NH_4-N. They believe the drop in pH either may have caused a direct physiological change, or it may have adversely affected CO_2 solubility and hence, availability. Pratt and Fong (53) and others point out that ammonia utilization results in a rather sharp drop in pH level; whereas nitrate utilization brings about a rise in pH level. Chu (54) demonstrated that at proper concentrations of NO_3 and NH_4, algae of the types he studied grew equally well on NH_4-N and NO_3-N. However, he notes that the optimum range (2-13 mg/1) was narrower for NH_3N than for NO_3-N (0.3-17 mg/1).

Although with the maintenance of a suitable pH level, algae apparently grow equally well regardless of whether NO_3 or NH_4 serves as the nitrogen source, there is evidence that NH_4 is assimilated in preference to NO_3. Thus, Pratt and Fong (53) observed than when NH_4 and NO_3 were supplied together, to *Chlorella vulgaris*, the pH level dropped at first, and then began to rise. This reaction indicates that first ammonia and then the nitrate was used. Using nitrogen-starved cells (*Chlorella vulgaris*), Syrett (55) found that the initial assimilation of ammonia N by the starved cells was about four times as fast as that of nitrate nitrogen. He observed an increase in respiration rate, cellular carbohydrate depletion, oxygen consumption, and CO_2 production following the assimilation of nitrogen, and that rate of assimilation of nitrogen, in turn, was increased by the addition of extracellular glucose. The respiration rate that followed the assimilation of nitrate was greater than that coming after the assimilation of ammonia (except for oxygen uptake due to the high oxygen content of nitrate). Syrett's observations indicate that the energy requirement and the enzymatic process for the assimilation of these two sources of nitrogen are totally different.

Urea constitutes an excellent source of nitrogen for all unicellular green algae. There are many advantages in the use of urea as a nitrogen source in the mass culture of algae, chief of which is the maintenance of a constant pH level despite an intensive uptake of urea-N. Another advantage is in the fact that heavy concentrations of urea are not inhibitory to algal growth (56).

In a study involving the culture of Chlorella grown on a variety of nitrogenous compounds, most of which are organic, Ludwig (57) found that 36 of them constituted good sources of nitrogen, and that growth on some was better than that on NO_3-N. However, the latter outcome may have been the result of pH level rather than availability of nitrogen. Saunders (58) reports that several species of autotrophic algae are able to utilize alanine, aspartic acid, aspargine, glutamic acid, glycine, and succinamide as sole sources of nitrogen. He also lists an extensive array of organic nitrogen compounds that are utilized by algae for energy and growth. Fogg (59) states that any substance which is an intermediate in nitrogen metabolism may be available as a nitrogen source for algae provided it can be absorbed into the algal cell.

As pointed out by Syrett (51, 55), the first step in the assimilation of NH_4-N by an algal cell is incorporation of the nitrogen into an amino acid. This is accomplished through the transformation of ammonia into ketoglutaric acid, followed by a further reductive amination to glutamic acid, and eventually to glutamine and alanine, the first of the amino acids to be produced. No information is available regarding enzymatic activity concerned with permeability or enzymatic processing of NH_4-N prior to the initiation of amino acid formation.

Urea-N probably is incorporated directly, i.e., without a prior breakdown to NH_4-N, in a pathway similar to that followed in the assimilation of NH_4-N, as is evidenced by the lack of urease in *Chlorella spp.* This is accomplished by way of a direct combination of urea with ornithine to form arginine. Such a direct mechanism could account for the difference which exists between the rate of assimilation of N by nitrogen-starved cells in the presence of NH_4-N and that in the presence of urea (51, 55).

A chain of consecutive enzymatic activity is required in the incorporation of NO_3-N into cellular material. Included in this chain is the enzyme nitrate-permease, an enzyme required for the "pumping" and penetration of the nitrate ion into the cell (60). Once in the cell, a succession of reductase enzymes take part in the reduction of NO_3 ion to a form ready for incorporation into an amino acid.

Nicholas (60) proposed a mechanism model that includes four consecutive enzymatic steps as follows:

$$HNO_3 \xrightarrow[2\,[H]]{\text{nitrate reductase}} HNO_2 \xrightarrow[2\,[H]]{\text{nitrite reductase}} H_2N_2O_2 \xrightarrow[2\,[H]]{\text{hyponitrite reductase}} NH_2OH \xrightarrow[2\,[H]]{\text{hydroxylamine reductase}} NH_3$$

It has been suggested that the last step be omitted, since organic-N may be formed directly from hydroxylamine (51). The mechanism proposed by Nicholas could explain the differences which exist between assimilation and removal rate of nitrate and those of other more reduced forms of nitrogen, and why it is possible that a unique kinetic relationship can exist for each major source of nitrogen. Ideally, if the enzyme kinetics of the above mechanism were known, a more rational kinetic model could be formulated regarding the function of algal growth with respect to nitrate concentrations. However, the presence in many algae (including Chlorella) of the special enzyme, as reported by Nicholas (60), nitrate permease, which functions in the transport of nitrate through the cell wall together with its unique characteristics (one of which is its induction in the presence of NO_3^-) make a very complex mechanism for the basis of a kinetic model. The complexity is magnified by the need to account for the many reductase enzymes in developing such a model.

The presence of a typical nitrate-permease enzyme strongly influenced in its activity by the genetic make up of the cell, as is proved by the existence of many mutants, may serve to explain the adaptation of various algal species and varieties to a wide range of nitrate concentrations. Some marine algae for example can attain almost full growth at NO_3-N concentrations of 0.01 mg/l or less (61); whereas many algae indigenous to oligotrophic lakes require concentrations of N of at least 0.1 mg/l. Many freshwater algae adapted to eutrophic conditions require nitrogen concentrations in excess of 1 mg/l (62).

Some species of blue-green algae (e.g., species of Anabaena, Cylindrospermum, and Nostoc) are capable of fixing atmospheric molecular nitrogen when other sources of nitrogen are not available or their concentrations are extremely low (62, 63). The unavailability of other sources is a prerequisite for fixation. In fact Fogg (63) stated that the presence of ammonia and urea completely inhibits nitrogen fixation, as does nitrate for those algae adapted to the assimilation of nitrate-nitrogen. Few blue-green algae other than those above named, are capable of fixing nitrogen, although there is the possibility that some may be able to do so at a much reduced rate. Even among those planktonic blue-green algae indigenous to waters having rather low inorganic nitrogen concentrations, such as the Oscillatoriaceae, the number showing any evidence of being able to fix molecular nitrogen is extremely limited (62). In his review, Fogg (63) also states that part of the fixed nitrogen may be liberated as extracellular nitrogenous substances, especially by young cells. The immediate utilization of this soluble nitrogenous substance by non-nitrogen-fixing algae is quite likely. Fogg states that there is no satisfactory evidence to establish any nitrogen fixation by algae belonging to groups other than the blue-green algae, and that the mechanism of nitrogen fixation generally is similar to that of nitrogen-fixing bacteria.

Controversy still exists as to the quantitative significance of nitrogen fixation by phytoplankton in natural bodies of water. Genera of nitrogen-fixing algae are not particularly prevalent in algal blooms, and no information is available concerning heavy blooms in which nitrogen-fixing algae are predominant in natural bodies of water having a low concentration of nitrogen. On the other hand, on the basis of her experiments on growing nitrogen-fixing algae under optimum conditions, Allen (64) concluded that theoretically as much as 480 pounds of nitrogen could be fixed per acre per year.

The importance of nitrogen-fixation in algal mass cultures is quite limited owing to the prevailing nitrogen concentration in the solution and to the fact that nitrogen-fixing genera usually do not appear in such systems. In the course of our experiments, no blue-green algae whatsoever was used. In fact, most of the blue-green algae that were considered for mass culture belong to the Oscillatoriaceae family which apparently does not possess the ability to fix atmospheric nitrogen. The use of continuous culture chemostatic techniques for describing the kinetics of both nitrate and ammonia uptake by various marine phytoplankton was demonstrated by Caperon (65, 66), Eppley *et al.* (67), and McIsaac and Dugdale (68).

EXPERIMENT, DESIGN AND METHODOLOGY

Experimental Design

A series of five experiments were performed in which the specific growth rate of algae as a function of ammonia concentration was compared. In four of the experiments (Experiments 1 through 4) *C. pyrenoidosa* was tested at different temperatures (19°C, 28.5°C, 35°C and 39.2°C). Emerson strain of *C. pyrenoidosa* was used at the two lower temperatures (Experiments 1 and 2) while the high temperature strain of *C. pyrenoidosa* TX 71105, was used at the higher temperature (Experiments 3 and 4). The fifth experiment, (Experiment 5), was designed to show the relationship between specific growth rate and ammonia concentration for a mixed culture of algae at a constant temperature of 25°C. In each experiment a series of runs were made, each at a different nitrogen level so as to develop a curve for specific growth rate (or dilution rate) versus ammonia concentration remaining unassimilated and still in solution.

Experimental Organisms

Two strains of the species *Chlorella pyrenoidosa*, TX 71105 and Emerson were selected as the major groups of experimental organisms. Strain TX 71105 was used in experiments 1 and 2 in which the temperature was kept at 35°C and 39.2°C respectively. The Emerson strain was used in Experiments 3 and 4 in which the temperature was maintained at levels of 28.5°C and 19°C respectively.

The reason for selecting the species *C. pyrenoidosa* were (a) its rapid growth rate; (b) its ubiquity in nature, particularly in polluted receiving bodies of water and in algal waste treatment ponds; (c) the experience gathered in growing the species under laboratory conditions in both photosynthesis research and mass cultures; (d) the availability of kinetic parameters concerning its growth with respect to irradiance.

Adaptation of the Chlorella pyrenoidosa strains used in the experiments at low concentrations of nitrogen did not enter into the decision on the types to be used. In fact, the original isolation of Chlorella pyrenoidosa was from moist rich soils apparently having a high nitrogen content, while the inocula used in the present studies were obtained from stock cultures maintained on nitrogen-rich media over a number of years. However, since species of Chlorella most likely will be a major organism in practical algal systems, the influence of nitrogen availability and concentration of nitrogen on their growth and production rates is a very important consideration in the rational design of such systems.

In Experiment 5 a culture of algae consisting of a mixture of groups indigenous to the San Joaquin (California) drainage channels was used in making a comparison between the kinetics of "wild" types of algae and those of the laboratory strains used in the present study. The mixed culture consisted mainly of green algae, of green flagellate algae, and of diatoms. Among the genera were Scenedesmus, Ankistrodesmus, Pandorina, Gonium, Melosira, Cyclotella, Tabellaria, Asterionella, Navicula (69).

Pure cultures of the two strains of Chlorella pyrenoidosa were obtained from the Biodynamics Laboratory of the University of California (Berkeley) and from the U.S. Air Force School of Aerospace Medicine (AFSC). Stock cultures were kept on agar slants under bacteria-free conditions. However, no special precautions were taken to maintain the cultures free of bacteria while growing in the continuous growth chambers. The stock cultures were subcultured at four-week intervals to maintain the genetic characteristics.

Algal Growth Units

Four continuous chemostatic growth units and two continuous turbidostatic growth units were employed in the study.

Chemostatic Growth Units. Except for certain minor modifications, the five chemostatic growth units used in the experiments were the same in design and operation as those described by Oswald (70-72). They were constructed of cylindrical Pyrex culture tubes 70 cm in length and 5.5 cm inside diameter, culture volume of the individual tubes was approximately one liter. Each tube was equipped such that a predetermined dose of medium could be injected into it once each 30-minute interval throughout the course of an experimental run. The contents of the tube were maintained in a continuously stirred condition (continuously stirred tank reactor conditions--CSTR) by vigorously bubbling through the mixture of CO_2 and air.

Constant and continuous light intensity was applied unilaterally to each culture by four (per culture) 30-watt full-spectrum flourescent lamps.[a] To provide light saturation conditions throughout the cross section of the culture, the suspended solids concentration of the culture usually was kept at less than 130 mg/l. Light saturation was attained by imposing conditions which would result in the irradiance penetrating through the culture to be equal to or more than 0.05 cal/cm^2-min.

The temperature of the cultures in the units was maintained at the desired level (± 0.7°C) by passing water held at a suitable temperature through a "cold finger" inserted in each tube.

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[a] "Optima" type manufactured by the Duro-test Co., North Bergen, N.J.

Each tube was equipped with two outlets such that it could be operated as a turbidostatic growth unit when desired. Advantage was taken of this feature in the initial period of runs in which the conditions to be imposed were such that an inordinate length of time would be required for a culture to reach "steady-state" if grown under chemostatic conditions.

A schematic sketch of the units and their appurtenances are shown in Figure 1.

Turbidostatic Growth Unit. A turbidostat is characterized by the maintenance of a steady-state, predetermined population density accomplished by means of a photoelectric control. The photoelectric system regulates the population density by controlling the frequency and volume of dosage of medium and hence the dilution rate as determined by the amount of light penetrating a sample of the culture. A turbidostatic control system has marked advantage over a chemostatic control system with respect to the time required to reach steady-state conditions following a change in the experimental conditions. It also permits a greater degree of accuracy and precision than is possible with a chemostatic control. To obtain a high degree of accuracy, a turbidostat should be constructed specifically according to the experimental needs.

The turbidostatic growth unit system used in the study reported herein consisted of a turbidostat and a growth vessel. The turbidostat and the vessel controlled by it are diagrammed in Figure 2. The vessel was identical in design with that of the vessels used in the chemostatic system. In fact, they were interchangeable. The turbidostat, a two-channel unit, was assembled and constructed by the personnel of the Lawrence Radiation Laboratory of the University of California spcifically for work with unicellular algae in photosynthetic studies. One of the channels was used

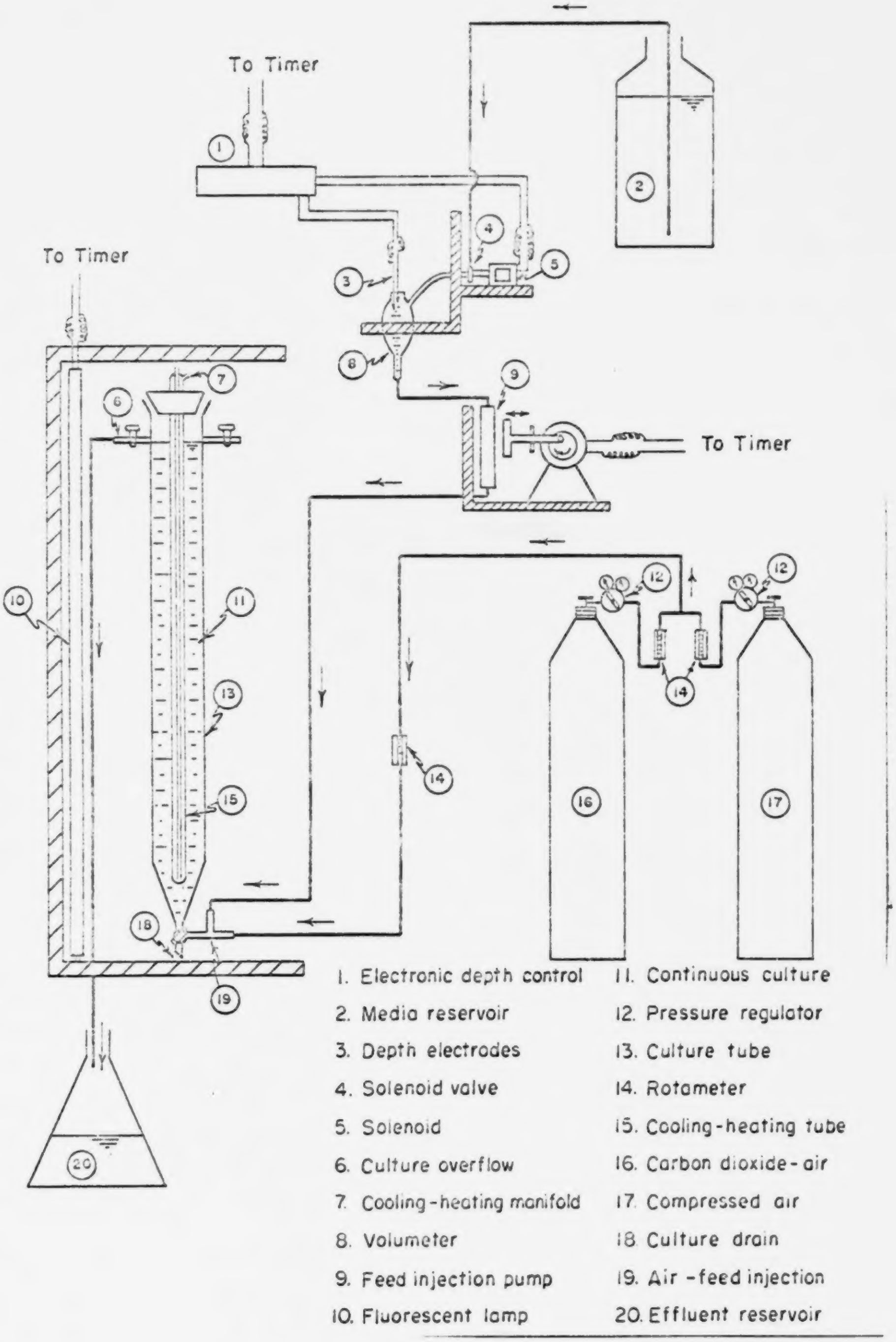
To Timer
To Timer
To Timer
1. Electronic depth control
2. Media reservoir
3. Depth electrodes
4. Solenoid valve
5. Solenoid
6. Culture overflow
7. Cooling-heating manifold
8. Volumeter
9. Feed injection pump
10. Fluorescent lamp
11. Continuous culture
12. Pressure regulator
13. Culture tube
14. Rotameter
15. Cooling-heating tube
16. Carbon dioxide-air
17. Compressed air
18. Culture drain
19. Air-feed injection
20. Effluent reservoir

FIGURE 1. DIAGRAMMATIC SKETCH OF THE CHEMOSTATIC GROWTH UNIT SYSTEM

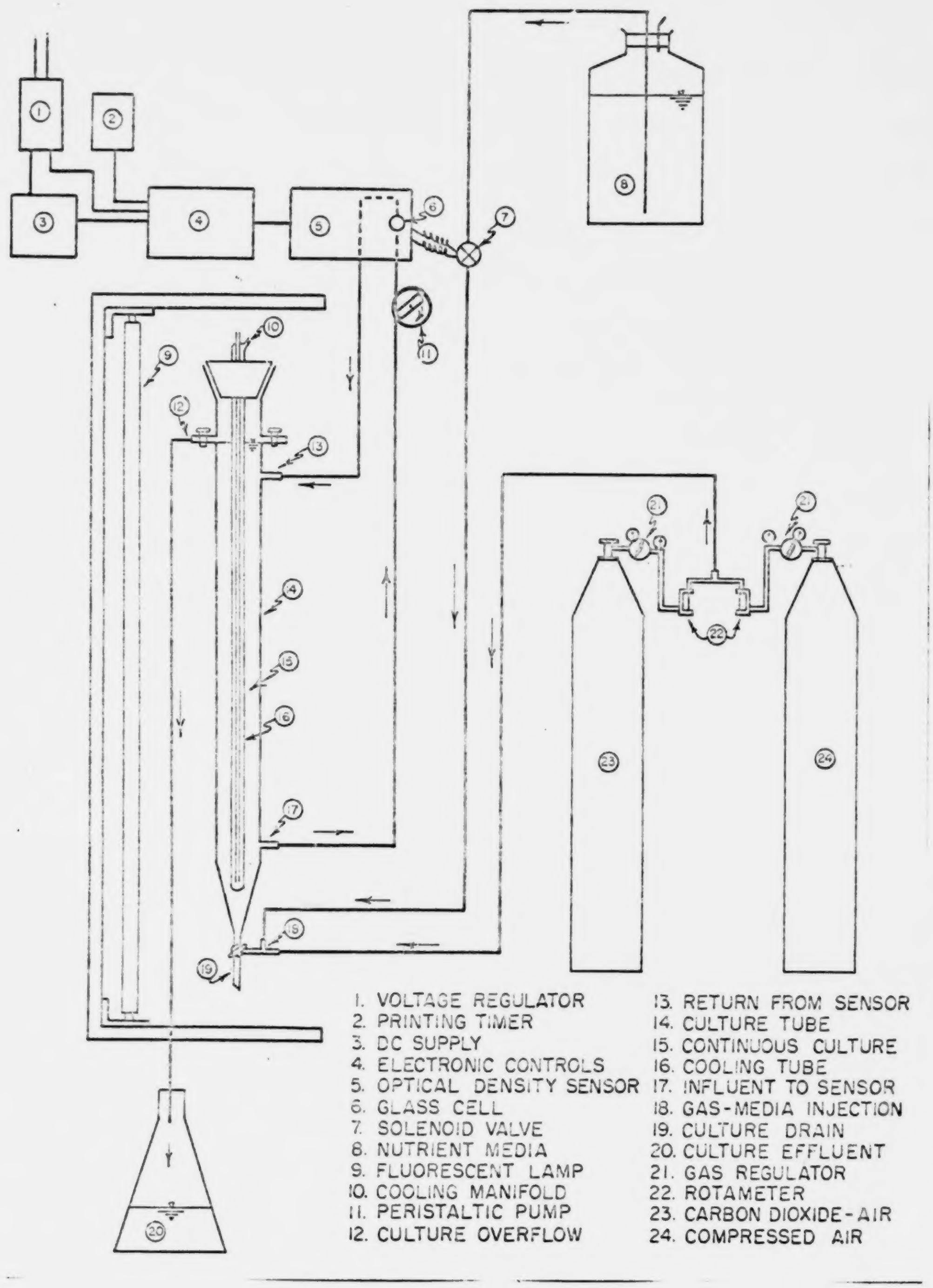
1. VOLTAGE REGULATOR
2. PRINTING TIMER
3. DC SUPPLY
4. ELECTRONIC CONTROLS
5. OPTICAL DENSITY SENSOR
6. GLASS CELL
7. SOLENOID VALVE
8. NUTRIENT MEDIA
9. FLUORESCENT LAMP
10. COOLING MANIFOLD
11. PERISTALTIC PUMP
12. CULTURE OVERFLOW
13. RETURN FROM SENSOR
14. CULTURE TUBE
15. CONTINUOUS CULTURE
16. COOLING TUBE
17. INFLUENT TO SENSOR
18. GAS-MEDIA INJECTION
19. CULTURE DRAIN
20. CULTURE EFFLUENT
21. GAS REGULATOR
22. ROTAMETER
23. CARBON DIOXIDE-AIR
24. COMPRESSED AIR

FIGURE 2. DIAGRAMMATIC SKETCH OF THE TURBIDOSTATIC GROWTH UNIT SYSTEM

exclusively for the turbidostatic system. The second was used interchangeably and temporarily with one or the other of the vessels of the chemostatic system, as was described previously.

In preliminary experiments it was found that monitoring of scattered light provided a better control of algae concentration in terms of weight of biomass (i.e., concentration as mg material per liter of volume) than did that of optical density. Consequently, scattered light sensor was used in the experiments to be described. An optical density sensor is strongly influenced by the pigment content of the biomass (primarily chlorophyll) while a scattered light sensor is more reactive to the concentration of biomass as expressed by total suspended solids or to the number of cells per unit of volume. However, the latter is somewhat influenced by the cell size distribution. During the experiments, the concentration of algal biomass in the turbidostatic units was maintained at 80 mg/l (dry basis) and variation was less than 10%.

A block diagram of the turbidostatic system used in the study is shown in Figure 3 and the components of the sensor, in Figure 4.* The sensor as set up to respond to optical density consists of a light probe, light source, a set of lenses, mirrors, light chopper, a sliding reference grating, phototube, and related switches, relays and other equipment. A light beam transmitted through an algal suspension flowing through the light probe passes through an infrared filter, a color filter (to transmit red light only), an amber-colored glass before being received by the photocell. The sensor was made to respond to light scattering by removing filters

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*In Figure 22 of the 1968 Kinetics Report (45), a part of the subtitle is missing (due to an editorial error). The subtitle should be:

Figure 22. Optical Density Sensor. A - light source; B - lens system; C - light chopper; D - glass probe insertion area; E - filters; F - photocell; G to K - from surface mirrors; L - sliding grating; M - control for grating.

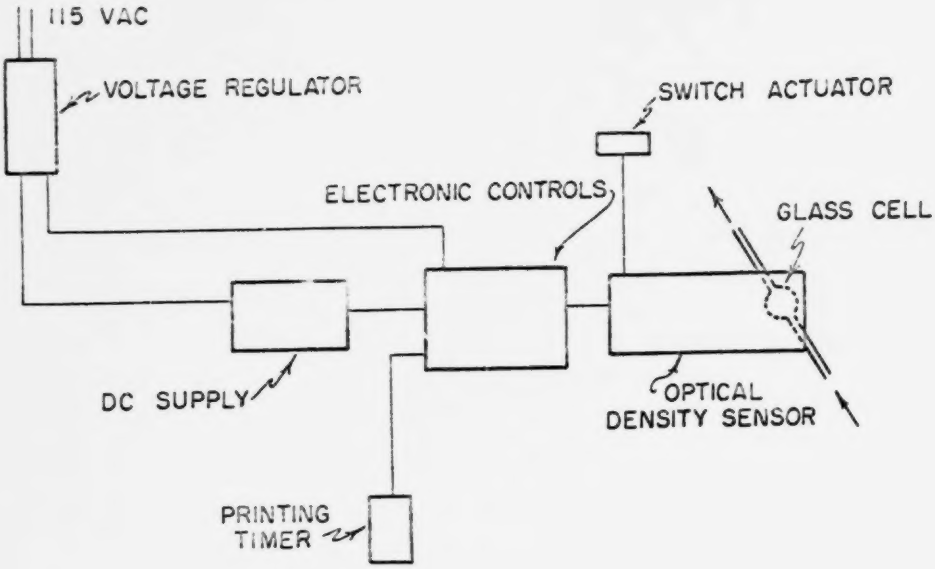
115 VAC
VOLTAGE REGULATOR
SWITCH ACTUATOR
ELECTRONIC CONTROLS
GLASS CELL
DC SUPPLY
OPTICAL DENSITY SENSOR
PRINTING TIMER

FIGURE 3. BLOCK DIAGRAM OF DENSITY REGULATING SYSTEM

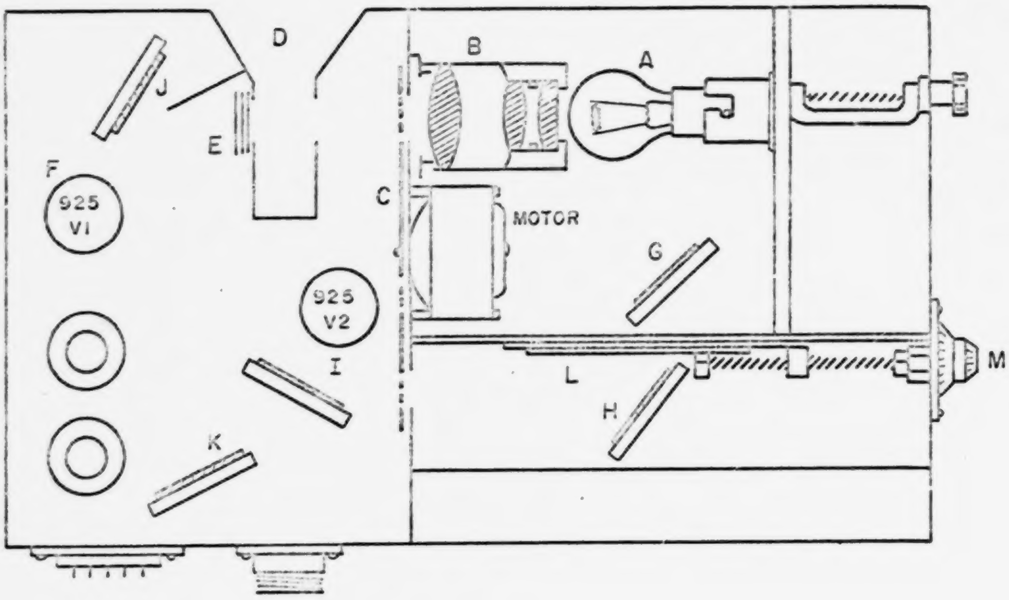
D
B
A
J
E
F
925 V1
C
MOTOR
G
925 V2
I
L
M
H
K

FIGURE 4. OPTICAL DENSITY SENSOR

A - light source; B - lens system; C - light chopper; D - glass probe insertion area; E - filters; F - photocell; G to K - from surface mirrors; L - sliding grating; M - control for grating.

and reorienting a shield. The glass probe is made of glass tubing flattened at the middle portion such that the probe assumes a pancake-like configuration having two flat parallel surfaces.

In operating the sensory system, a portion of the algal culture is passed continuously through a glass probe inserted in the optical sensor head. As soon as the photo diode senses a preset density in the probe, a switch and solenoid valve is activated. The solenoid valve opens and fresh nutrient is added to the culture, thereby diluting it. When the preset density is reached, the solenoid valve closes again. In using conventional growth vessel, algae culture is circulated through the glass probe by means of a peristaltic pump.

Media. The SERL Medium (Table 1) is a modification of Myers Medium (73). Its composition was extensively discussed in Reference (45) and was used in the course of these experiments with ammonia-sulfate substituting for potassium nitrate. Potassium was present in excess in the medium since potassium hydrogen phosphate was used as both a buffer and a phosphorus source. In order to eliminate any trace quantities of nitrate, $CaCl_2$ was substituted for $Ca(NO_3)_2$ in the medium. The composition of micronutrients is given in Table II.

CO_2 Supply. Air enriched with CO_2 (1%) was supplied to the chemostatic and turbidostatic units. Gas was supplied in excess in order to provide proper mixing and to insure an adequate supply of CO_2. Adequacy of CO_2 was checked by alkalinity tests and daily pH determinations.

Sampling. Samples were taken daily from each growth unit for the determination of various solids measurements, nitrogen and chlorophyll concentrations, and temperature. Samples were taken twice each day on those days in which steady-state conditions were apparently about to be reached.

TABLE I

COMPOSITION OF SERL AMMONIA MEDIUM

Nutrient	Molar Concentration in Media moles/liter	Preparation	
		Stock Solution moles/liter	Amount ml/liter
$(NH_4)_2\ SO_4$	(*)		
KH_2PO_4	0.6×10^{-2}	1.0	6.0
K_2HPO_4	0.3×10^{-2}	1.0	3.0
$MgSO_4 \cdot 7H_2O$	1.0×10^{-2}	1.0	10.0
Fe Versenol 120	1.74×10^{-4}	0.1	1.74
Micronutrients	(See Table II)		

(*) Ammonia concentrations varied according to experimental needs.

TABLE II

FORMULA OF THE MODIFIED ARNON'S MICRONUTRIENT STOCK SOLUTION (74, 75)

Nutrient	Molar Concentration in Media moles/liter	Preparation gr/liter in stock solution
H_3BO_3	4.6×10^{-5}	2.86
$MnCl_2 \cdot 4H_2O$	9.2×10^{-7}	1.81
$ZnSO_4 \cdot 7H_2O$	7.7×10^{-7}	0.222
$CuSO_4 \cdot 5H_2O$	3.2×10^{-7}	0.079
MoO_3 (99.5%)	1.0×10^{-7}	0.015
$C_oCl_2 \cdot 6H_2O$	1.7×10^{-7}	0.040

Medium contained in the medium reservoir and cultures in the growth units were sampled twice each week for determination of alkalinity. Total chemical analyses of the medium was made occasionally to check on the continued presence of nutrients in optimal concentrations. The size of the samples ranged from 50 to 150 ml. The effect of removal of the samples on the reactor material balance was taken into consideration in all cases.

Analysis

Solids Determination. The concentration of total dissolved solids, total suspended solids, and volatile suspended solids were routinely determined. The analyses were made according to SERL Analytical Methods (76). Algal concentration was assumed to be represented by the concentration of the total suspended solids, since inorganic media were used exclusively. This assumption proved to be justified since the ash concentration of the suspended solids remained within the range of 5.2 to 7.6%--a concentration characteristic of algal organic matter produced at a pH near 7.0. The (J) valve for the energy content of the algal cells (5.5 Cal/mg cellular material) used in the kinetic models is based on the assumption of low ash.

Nitrogen Determination. Nitrogen determinations were made of the total sample, of its soluble fraction, and of the suspended material (mainly algae and some bacteria) for cross-checking of the results. Nitrate-nitrogen was determined in order to detect any transformation of the reduced forms of nitrogen to nitrites and nitrates by the phenoldisulfonic acid method (77), whenever no chlorides were present in the media, and by the brucine method (77) when significant chloride concentration were present. Nitrite-nitrogen was determined by the diazotization method (77). Since nitrite-nitrogen in detectable concentrations rarely was present, nitrite determinations were made only infrequently. Ammonia and total unoxidized nitrogen determinations were made according to the A.O.A.C. version of the Kjeldahl method (78).

Chlorophyll Determinations. Chlorophyll content is an indicator of the condition of the algal system, especially in relation to nitrogen starvation. Chlorophylls A and B were determined according to the McKinney method (79).

Criteria for Steady State Conditions

Steady-state conditions were considered to have been attained when the dilution rate $(\frac{Q}{V})$*, algal concentration in the reactor and effluent (C_c), nitrogen concentration in the reactor and effluent (S_{N_1}), and nitrogen cellular content (N_c) were ascertained as remaining constant within a certain level for at least three consecutive days. Obviously the nitrogen levels S_{N_1} and N_c were determined only in those experiments involving nitrogen. The level of permissible variation depended upon the type of growth unit in use. A maximum variation of ± 5.0 percent was allowed for the turbidostatic units, and ± 6.0 percent for the chemostatic growth units. With each change in growth conditions, it took an average of 42 hours for the culture to reach steady-state in the turbidostatic unit and in chemostatic units operated as turbidostatic units at the start of a run. An average of 96 hours was required for steady-state conditions to be established in the chemostatic units.

Computations

The regression and correlation analysis of the logarithmic function of the modified exponential function developed as a part of the experiments involving nitrogen concentration as well as other statistical analyses were made with the use of an IBM 1620 Computer (cf. Appendix B).

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*All symbols used in this report are identified in Appendix A, Glossary.

EXPERIMENTAL RESULTS AND DISCUSSION

It can be shown that under conditions of constant concentration of algal biomass X_1 and with a completely mixed single-stage growth reactor operating at steady-state without cellular recycle, the net specific growth rate μ_n is equal to the dilution rate D_R (see reference (45), Chapter IV). The dilution rate is the reciprocal of the mean residence time θ which under the above conditions is practically equal to the mean cellular residence time θ_c.

also, $$\mu_n = \mu - K_d \qquad (2\text{-}1)$$

where μ is the specific growth rate, $\mu = \frac{dX_1}{X_1 \, dt}$, and K_d is the decay coefficient.

The specific growth rate μ is a function of the concentration of the growth limiting nutrient in the reactor (ammonia-nitrogen in our case) denoted by S_{N_1}.

thus, $$\mu = f\,(S_{N_1}) \qquad (2\text{-}2)$$

Since K_d can be assumed to be a constant under the given experimental conditions, it follows from Equations 1 and 2 that

$$\mu_n = f\,(S_{N_1}) \qquad (2\text{-}3)$$

The difference levels of S_{N_1} were achieved by varying the ammonia level for each of the experimental runs.

The results of the five experiments are tabulated in Table III and depicted graphically in Figures 5 through 9.

TABLE III

SUMMARY OF KINETIC DATA AND PARAMETERS FOR EXPERIMENTS ON ALGAL GROWTH WITH RESPECT TO AMMONIA-NITROGEN CONCENTRATION

Exp. No.	Algae Species	Temp. (°C)	μ_n (day^{-1})	K_A (mg/1 as N)	K_S (mg/1 as N)	S_{N_1} (mg/1 as N)
1	*C. pyrenoidosa* Emerson	19	1.36*	0.44**	0.27*	1.06*
2	*C. pyrenoidosa* Emerson	28.5	1.84*	0.67**	0.48*	1.75*
3	*C. pyrenoidosa* TX 71105	35.0	3.94*	0.96**	0.67*	2.16*
4	*C. pyrenoidosa* TX 71105	39.2	4.26*	2.12**	1.48*	4.88*
5	Mixed Algae (Indigenous)	25.0	1.24**	0.39*	0.23**	2.15*

*Derived directly from experimental data using graphical plotting.

**Derived from a regression analysis of the experimental data.

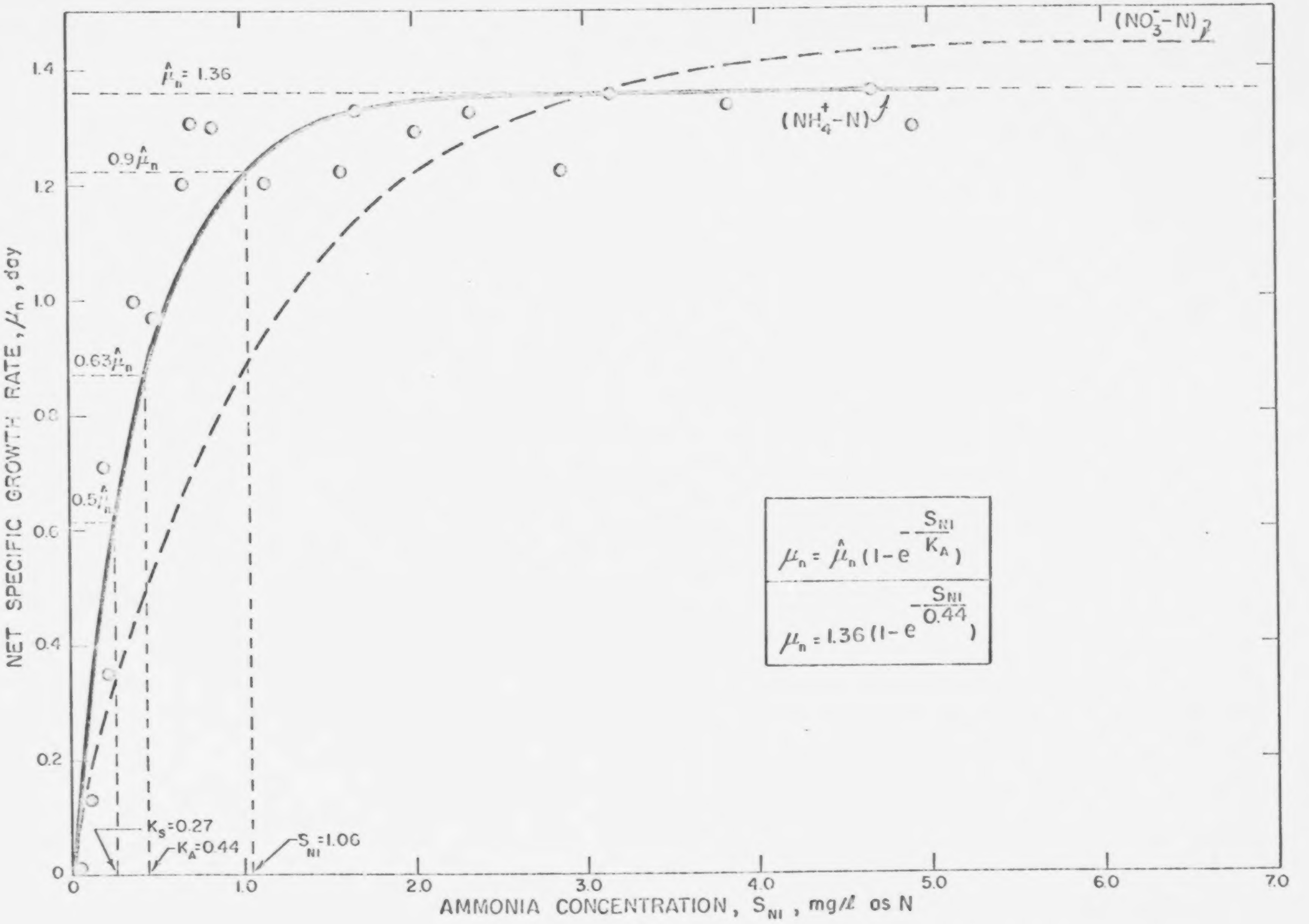

FIGURE 5. ALGAL NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION (EXPERIMENT 1 AT 19°C WITH *C. pyrenoidosa*, EMERSON STRAIN)

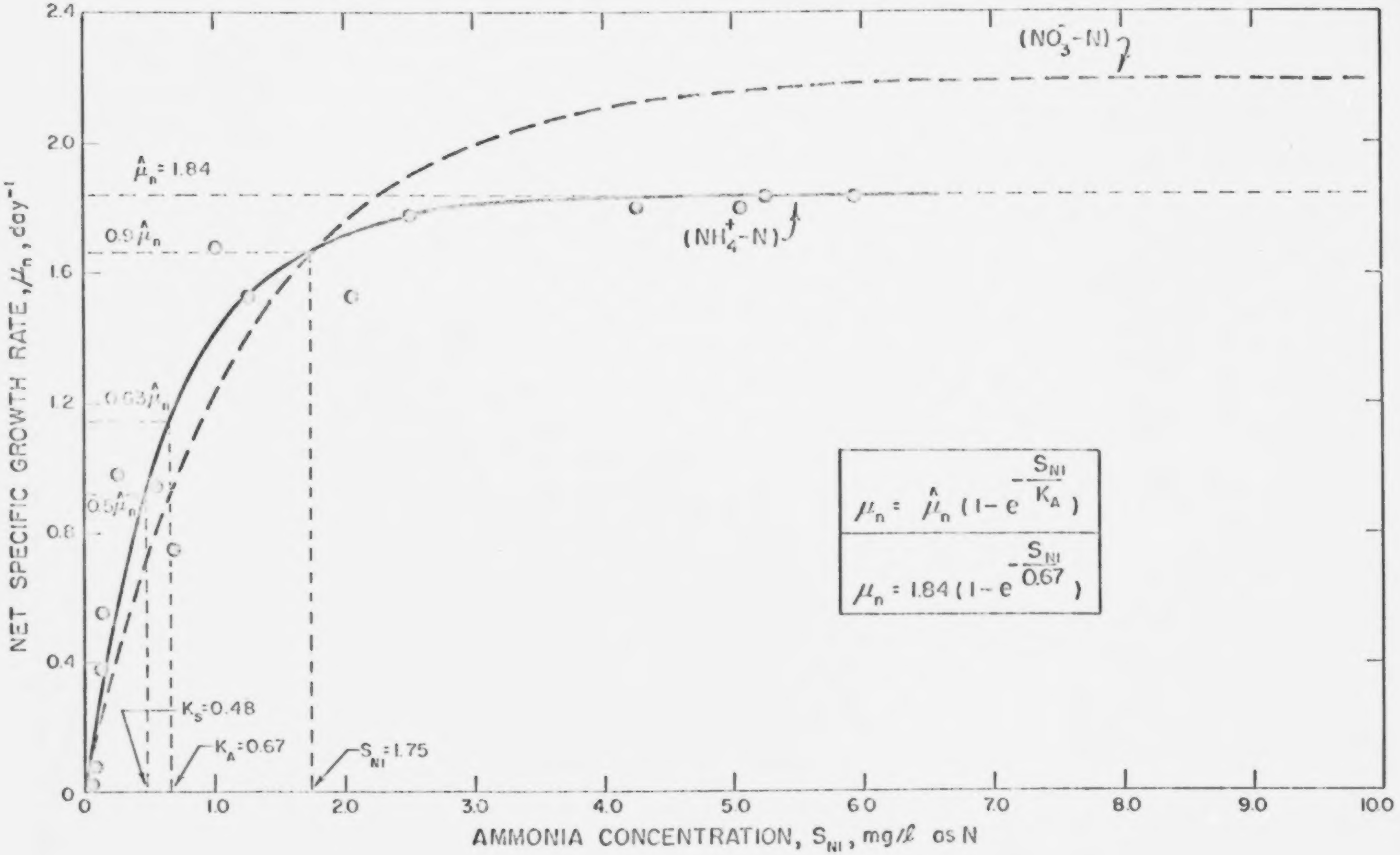

FIGURE 6. ALGAL NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION (EXPERIMENT 2 AT 28.5°C WITH *C. pyrenoidosa*, EMERSON STRAIN)

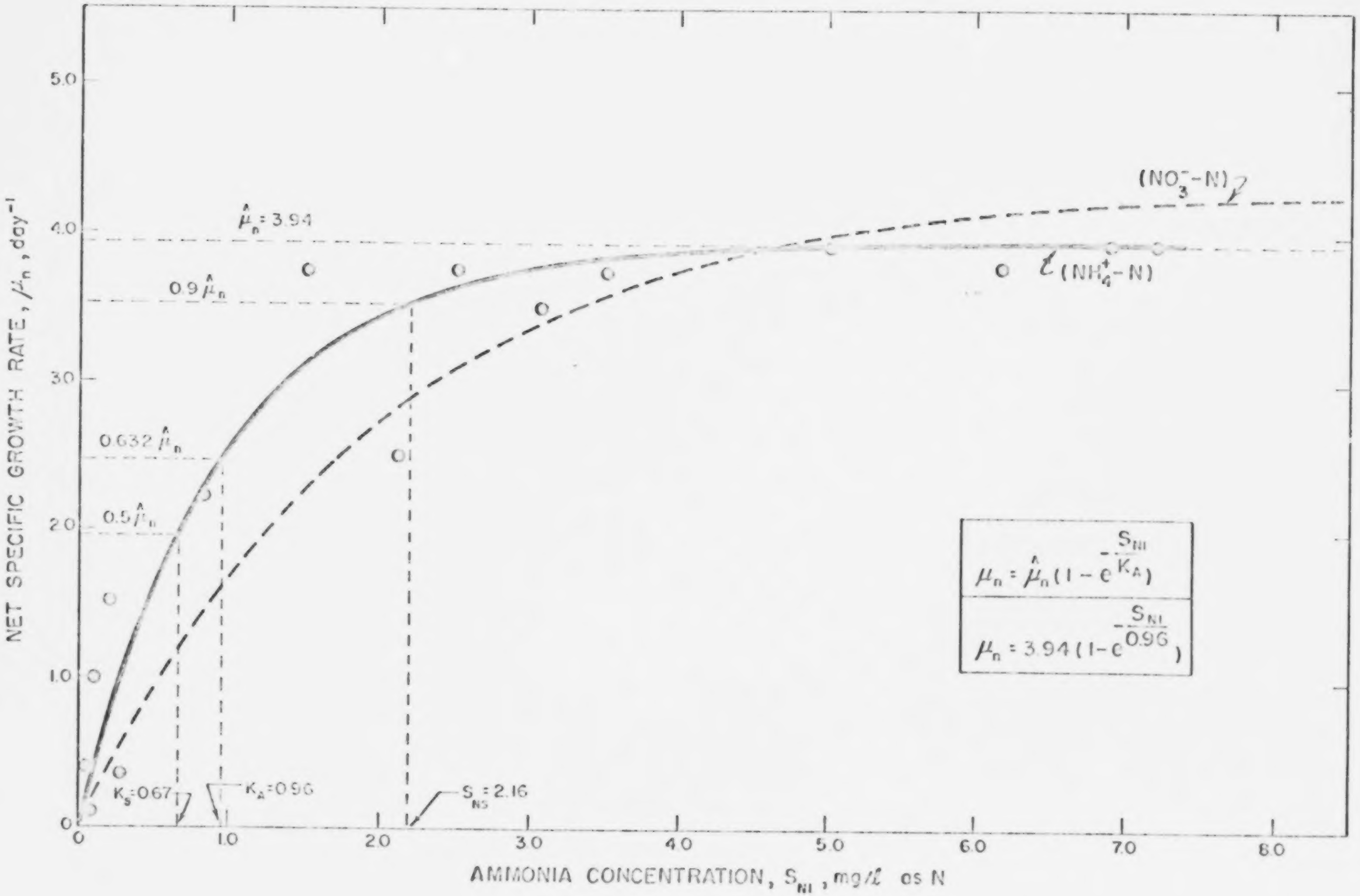

FIGURE 7. ALGAL NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION (EXPERIMENT 3 AT 35°C WITH C. pyrenoidosa 71105)

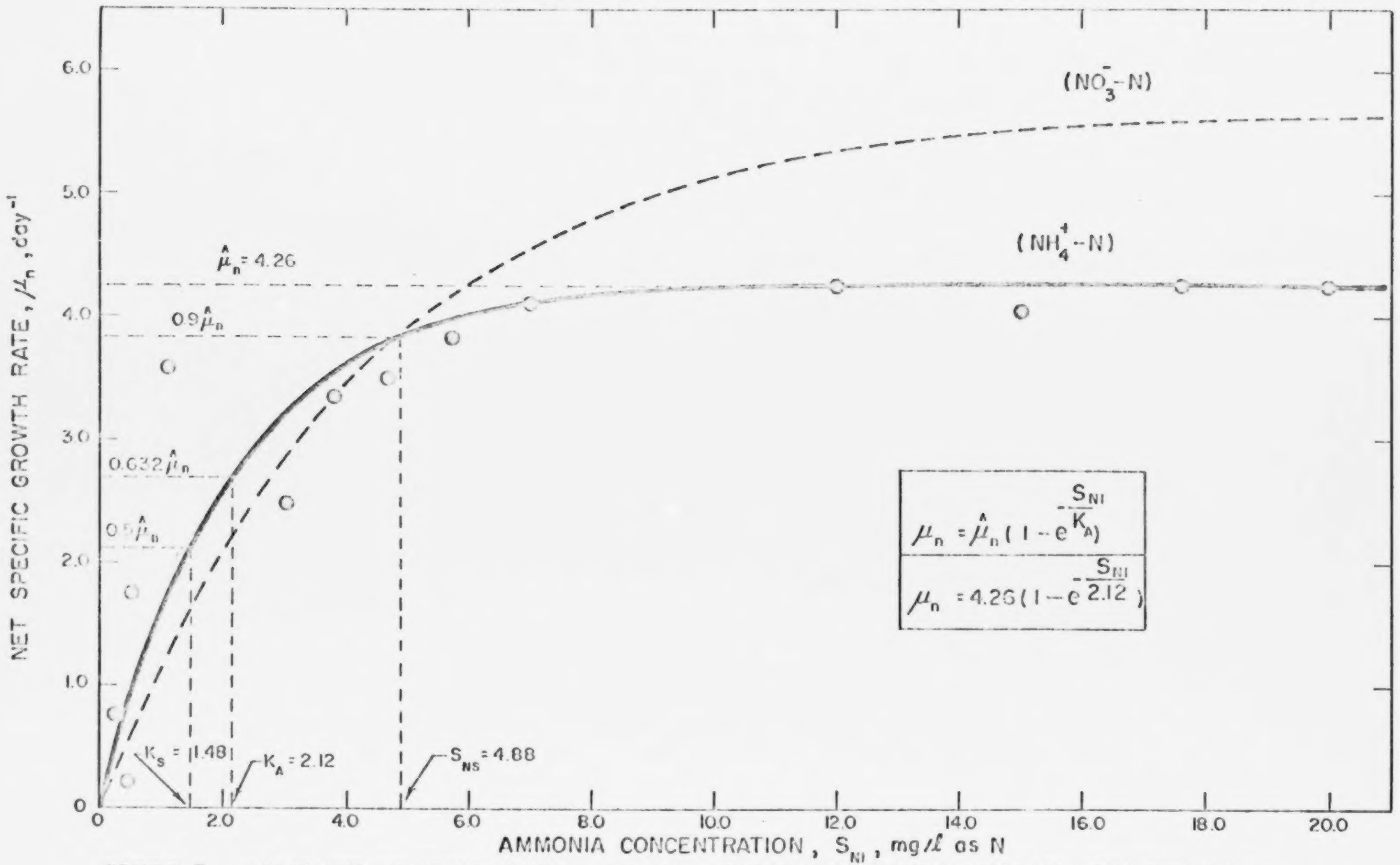

FIGURE 8. ALGAL NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION EXPERIMENT 4 AT 39.2°C WITH C. pyrenoidos 71105)

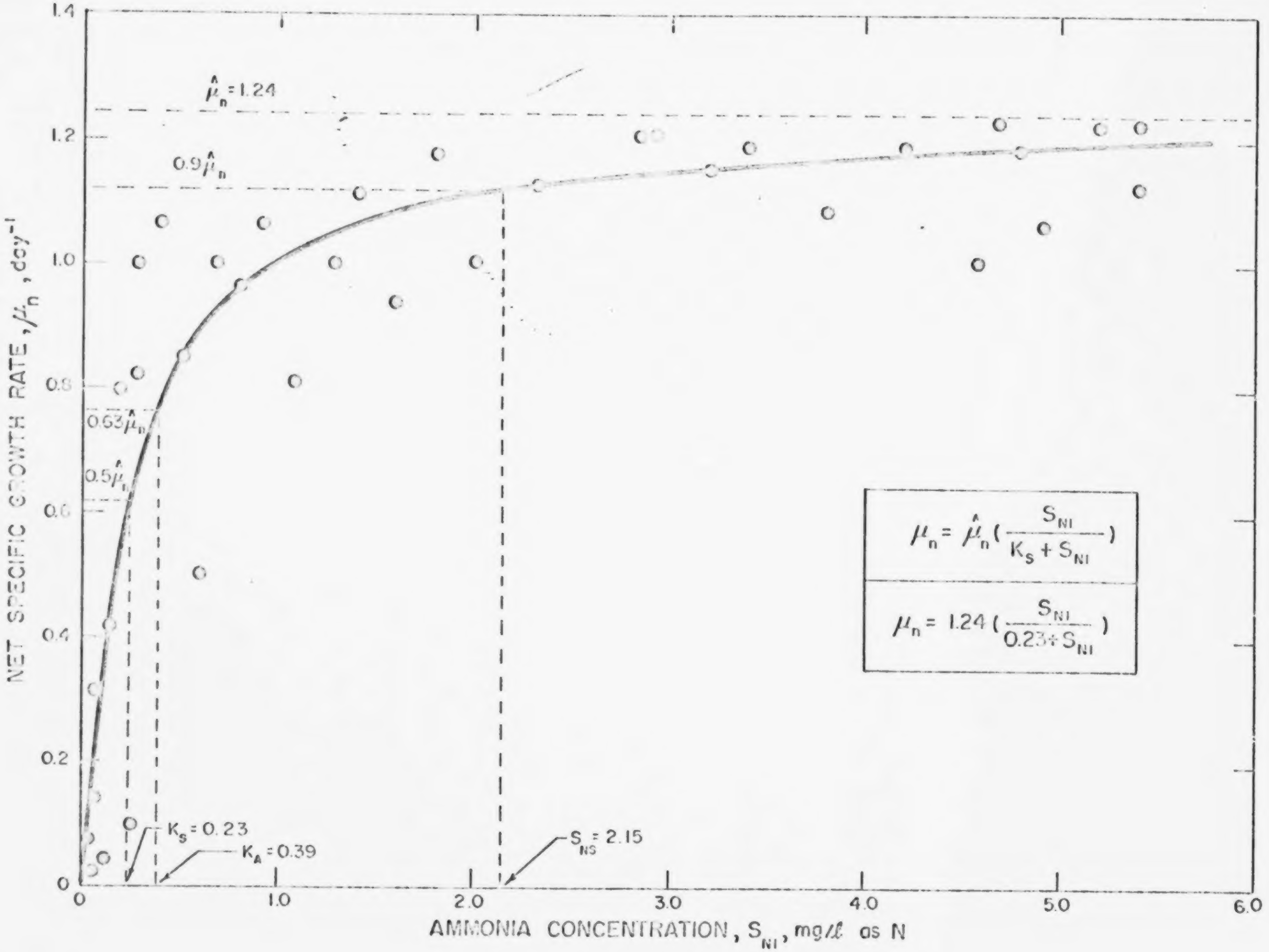

FIGURE 9. ALGAL NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION (EXPERIMENT 5 AT 25°C WITH MIXED CULTURE OF ALGAE INDIGENOUS TO SAN JOAQUIN VALLEY DRAINAGE WATER)

Experiments 1 through 4

From Table III and Figures 5 through 8, it can be seen that, while there is a definite relationship between μ_n and S_{N_1}, the plotted points were quite scattered and it was necessary to resort to a statistical analysis in order to draw conclusions with respect to a specific kinetic pattern. This same situation was found in previous experiments where nitrate-nitrogen constituted the nitrogen source (45).

Several major reasons for the scattering of the data points can be offered: 1) the use of six growth units simultaneously in long-term experimental runs with a consequent abundance of accumulated data; 2) the impossibility of avoiding the unplanned occurrence of minute fluctuations in growth conditions; 3) the inevitable variations in data based on biological functions; and 4) the consequent need to average the data.

The data points for net specific growth rate at the low nitrogen concentrations--the region of "nitrogen starvation"--in particular were so dispersed and inconsistent as to make it extremely difficult to draw any curve at all to fit them. The observed net growth rate varied from zero to approximately one-fourth the maximum net specific growth rate without apparent consistency. In a few runs growth at the lower nitrogen concentration was better than that at higher concentrations. This pattern of response differs from the classical pattern of growth curves of microorganism as functions of concentration and availability of energy source, e.g., bacterial growth in relation to organic substrate, and algae in relation to light. In the two latter cases, the data points plotted in the lower part of a growth response curve follow a clear and consistent pattern, whereas they have a tendency to become scattered at saturation levels.

Undoubtedly, a possible reason for the apparent inconsistency of the data is the relative inaccuracy attendant upon using analytical methods in measuring low concentrations, i.e., at the lower end of their range of sensitivity. However, this was not the major reason, since the inconsistencies occurred repeatedly in successive experimental runs. Perhaps genetic adaptation and natural selectivity exert a deciding influence. This could have a bearing on the results, since the strains of C. pyrenoidosa used in the study are not particularly adapted to low concentrations of nutrients. Another possibility is that while nitrogen starvation may not affect energy supply, it may result in damage to the protoplasm of the algal cell; and with increasing severity of starvation, to the chloroplasts, enzymatic structures, and the nucleic acids. Since the extent and affect of the damage differs from cell to cell, the net effect on the growth of the culture as a whole may vary from one experiment to another. A further complicating factor is that damaged cells are in a disadvantageous position with respect to bacterial competition, and are more susceptible to deficiencies in trace element supply.

The specific growth rate of algae at low nitrogen concentrations has an important bearing in studies of natural bodies of water undergoing a transition from oligotrophic to eutrophic conditions. Since the retention period in such bodies of water usually is relatively long, an accumulation of phytoplanktonic algae will occur if the net specific growth rate is only slightly greater than that of herbivorous decay, even though the rate is only a fraction of its value under optimum conditions. A parameter of importance in these cases is the critical concentration of nitrogen (or of any other essential nutrient), i.e., that concentration level below which no growth occurs. The critical concentration level, therefore, is the upper limit of nutrient concentration at which a body of water can remain oligotrophic.

The inconsistency of the data pertaining to the lower levels of nitrogen concentration poses no problem in the present study, since its primary concern is with the removal of algal nutrients by assimilation and with maximum algal growth and production. The latter are not practically done at low nitrogen concentrations. If information should be needed, an approximation of the concentration of nutrient at which growth is limited can be made even from the scattered data points in Figures 5 through 8.

In order to determine what relationships did exist between μ_n and S_{N_1}, a rigorous regression analysis of the data collected in Experiments 1 through 4 was performed. This analysis was performed with the aid of an IBM 1620 programmer (see Appendix B for the program used).

It was found that the function $\mu_n = f(S_{N_1})$ was best described by the exponential type curve.

Thus, for the above experiments, Equation (2-3) becomes:

$$\mu_n = \hat{\mu}_n \left(1 - e^{-S_{N_1}/K_A}\right) \qquad (2\text{-}4)$$

in which $\hat{\mu}_n$ is the maximum net specific growth and its value can be determined directly from Figures 5 through 8 once S_{N_1} levels reach saturation and the curve becomes parallel to the abscissa.

K_A is a constant and is equal to the value of S_{N_1} corresponding to $0.63\,\hat{\mu}_n$. Its exact value can be determined from the experimental data by the regression analysis of the function $\log \dfrac{\hat{\mu}_n}{\hat{\mu}_n - \mu_n} = \dfrac{0.434}{K_A} S_{N_1}$.

The exponential expression of Equation (2-4) was found to describe best $\mu_n = f(S_{N_1})$ in Experiments 1 through 4. Its form differs slightly from Michaelis-Menten type expression $\mu_n = \hat{\mu}_n \left(\dfrac{S_{N_1}}{K_S + S_{N_1}}\right)$ which is used extensively to express the relationship between specific growth rate μ and growth limiting substrate S. In the Michaelis-Menten expression, K_S denotes the saturation constant which is equal to the value of S or S_{N_1} corresponding to $0.5\hat{\mu}$.

Experiment 5

In Experiment 5 a similar scattering of data points was observed. A regression analysis was also performed on this data and in this case the Michaelis-Menten type expression, which defines a rectangular hyperbola, was found to best describe the relationship between μ_n and S_{N_1}. Thus, the results in Experiment 5 can be described by:

$$\mu_n = \mu_n \frac{S_{N_1}}{K_s + S_{N_1}} \qquad (2\text{-}5)$$

Although the data points describing $\mu_n = f(S_{N_1})$ turned out to be quite dispersed, the regression analysis of the function

$$S_{N_1} = \mu_n \frac{S_{N_1}}{\mu} = K_s \qquad (2\text{-}6)$$

which is a linear derivation of Equation 5, was found to give the required parameters of μ_n and K_s of 1.28 day^{-1} and 0.23 mg/1 as N correspondingly. Equation (2-6) was used in the regression analysis rather than the reciprocal function of equation (2-5) due to the problems in least-square analysis of the reciprocal function described in Reference (45).

From Figure 9 it can be seen that the maximum specific growth rate attained in Experiment 5 was significantly lower than that in the experiments involving the use of C. pyrenoidosa (Experiments 1 through 4 . Since the elapsed time in this experiment was relatively short (6 weeks) it was unlikely that any genetic adaptation could have taken place which would have resulted in an increase in growth rate capacity to reflect the optimal laboratory condition under which the algae were cultured.

Two additional characteristics of the experiment are worthy of note: a) In a few of the runs, the response in the form of increased growth rate to minute increases in nitrogen concentration was quite rapid. Moreover, the maximum specific growth rate was approached at relatively low concentrations. Consequently, the value for S_{NS} was rather small as compared to that for the experiments with *C. pyrenoidosa*. b) The average yield coefficient was significantly higher than that obtained in the experiments with *C. pyrenoidosa* as is evident from the reduced nitrogen content of the algae in Experiment 5. Moreover, the extent of the increase in the yield coefficient with decrease in S_{N_1} was less than that in Experiments 1 through 4.

Experiment 5 should be regarded as being only preliminary in nature. It was conducted for the purpose of gaining some insight as to the growth rates of algae indigenous to waters having a low concentration of nitrogen, especially as compared to those of algae adapted to heavy concentrations of nitrogen. Certain limitations resulted from the nature of the experimental conditions serve to keep the experiment from transcending its classification as preliminary: a) The precise composition of the population of the inoculum and of the culture with respect to relative cell numbers of the constituent algal groups was neither determined at the time of inoculation nor during the course of the experiment. Towards the end of the experiment numbers of the genera *Scenedesmus* and *Ankistrodesmus* became the predominant organisms, while those of the green flagellates dropped sharply. The drop in number of diatoms was moderate, and was noticed mostly in those runs in which the heavier concentrations of nitrogen were used. b) As noted previously, the culture consisted of a mixture of algae collected from widely divergent sources, ranging from the diatoms *Tabelaria* and *Asterionella* and the green alga *Ankistrodesmus* which are indigenous to clear, relatively unpolluted waters having a low nitrogen content, to those green algae such as *Scenedesmus*, and green flagellates such as *Pandorina* and *Gonium*, which are found in waters of varying degrees of pollution ranging from moderate to heavily polluted and rich in nitrogen, usually of the unoxidized forms. The inoculums contained few if any blue-green algae. The blue-green algae are very common in blooms of phytoplankton which occur in natural bodies of water having a low nitrogen concentration. c) Although the conditions under which the indigenous algae were grown may have been optimum for *C. pyrenoidosa*, they may not necessarily have been so for the indigenous algae. For example, it is well known that the diatoms have a rather narrow temperature requirement. Other algal species differ among themselves with respect to light, physical, and nutritional requirements (30).

Comparison of Ammonia and Nitrate as Nitrogen Sources

In order to compare the relative effects of nitrate and ammonia on the specific growth rate of *C. pyrenoidosa*, data from Reference (45) was plotted in Figures 5 through 8 under the exact same light, temperature and nutrient conditions as used in the present study (only the nitrogen sources differed in the nutrient media used for the two studies). A number of interesting differences in the growth curves can be observed in these figures. These are as follows:

(a) In all four experiments the maximum specific growth rate was significantly lower when ammonia nitrogen was used as compared to the previous experiments with nitrate. This phenomenon can be explained by the adaptation of the specific strain used in the experiments to nitrate medium and/or due to the difficulties in maintaining optimal and constant pH levels in the case of ammonia. It might also be due to inhibition caused by the increased ammonia concentration which followed the increased specific growth rate;

(b) In all experiments, the ammonia-nitrogen concentrations corresponding to a given specific growth rate was significantly lower--or, in other words, the algae exerted a higher net specific growth rate under relatively lower concentrations of nitrogen in the ammonia form. This phenomenon can be explained by the direct use of the reduced forms of nitrogen (i.e., ammonia) by the algae without the necessity of resorting to the nitrate-reductase enzyme mechanism described previously which is an energy-consuming operation.

The same observations were also reported by Eppley (67) and by MacIsaac and Dugdale (68) who both studied nitrogen uptake in various marine phytoplankton. Both references indicate that uptake rates can be used to estimate specific growth rates. Thus, the values of K_S and K_A, both of which correspond to nitrogen uptake rates, would also correspond to specific growth rates. This point was emphasized by Strickland (81).

The values of the various parameters such as K_A, K_S, together with the value of S_{N_1} corresponding to a given value of μ_n at 1.0 day^{-1} are also given in Figure 10 where the data gathered in the experiments with NH_4^+ is compared with the data from the previous experiments with NO_3^--N. Thus, all the above parameters are significantly lower when ammonia-nitrogen constituted the limiting factor as compared to nitrate-nitrogen. It should be noted that the above differences were observed at all temperatures tested and the difference was more marked as the temperature was raised above 30°C with C. pyrenoidosa TX 71105.

The Effect of Temperature

Temperature effects on the relationship between ammonia-N concentrations and the net specific growth rate were quite similar to those observed in the experiments with NO_3^--N (45).

The ability of certain species of algae to grow even under extremely

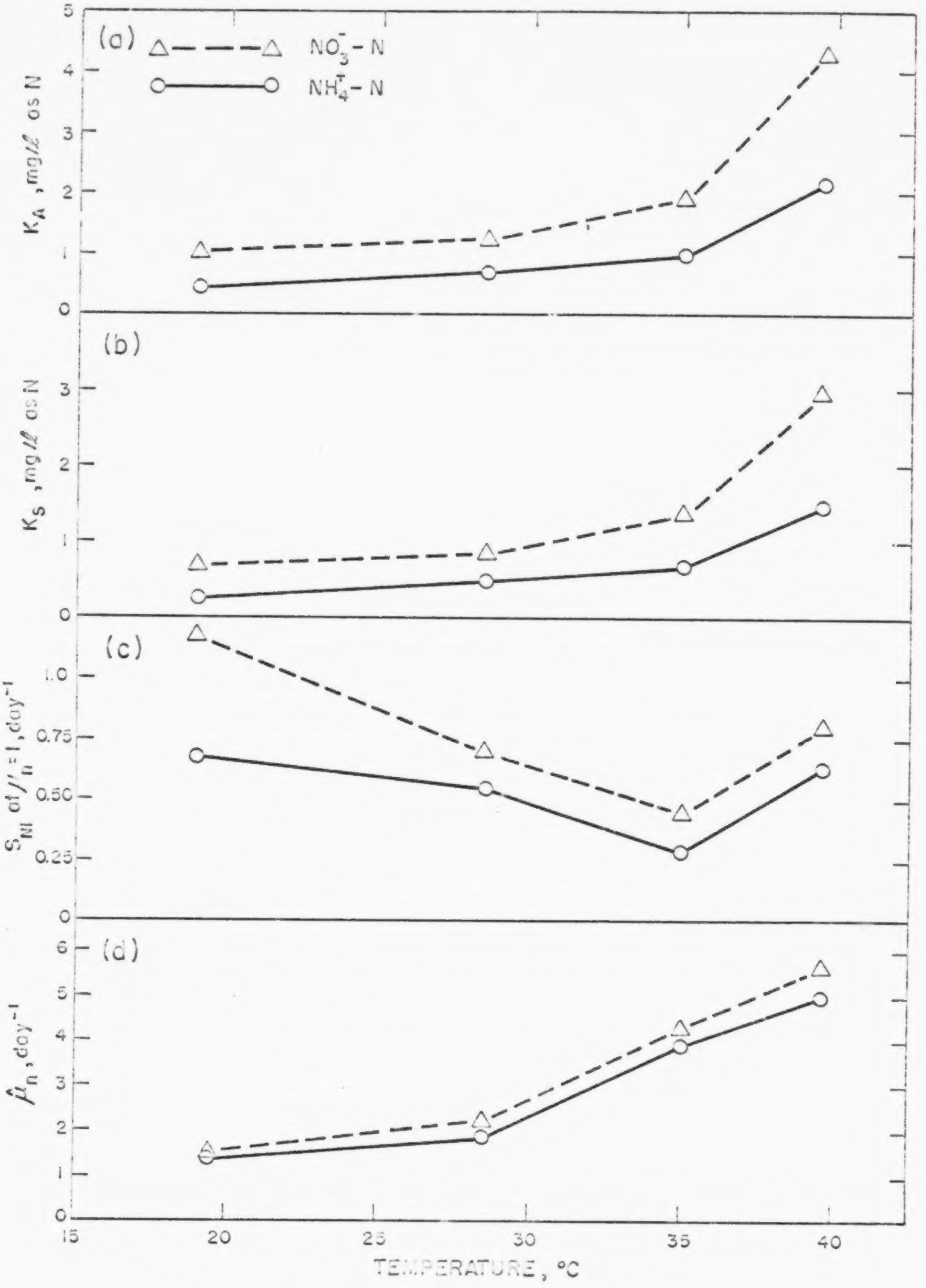

FIGURE 10. EFFECT OF TEMPERATURE ON VARIOUS KINETIC PARAMETERS WHEN EITHER AMMONIA OR NITRATE SERVED AS THE ALGAL GROWTH-LIMITING FACTOR

low nitrogen concentrations while other species are not adapted to grow under such conditions is evident from the results of Figures 10-12. Here data compiled by Caperon (66) on the marine armored flagellate *Isochrysis galbana*, together with data compiled on other marine phytoplankton (67) and data reported in experiments using *Selenastrum gracile* (7, 8), are compared with the data gathered in experiments from the previous study (45) with NO_3-N and in the current studies with NH_3-N. Interestingly, a substantial growth rate of approximately 0.5 day^{-1} took place with *I. galbana* at a nitrogen concentration as low as 0.02 mg/l (20 μ mg/l).

The results depicted in Figures 10-12 demonstrate the importance of genetic adaptation with regard to nitrogen uptake by various algae species and strains as well as the growth of the various alga with respect to nitrogen concentration.

The effect of the temperature on the various growth kinetic parameters of *C. pyrenoidosa* is shown by a family of curves in Figure 10 and Table III while its effect on the net specific growth rate is shown in Figures 11 and 12.

While the maximum specific growth rate increased substantially with temperature (sec. (D) in Figure 10), sections (A) and (B) in Figure 10 indicate that this was also followed by increased concentrations of nitrogen which were required to support such an increased growth rate. On the other hand, as indicated by sections (C) of Figure 10, the concentration of nitrate-nitrogen corresponding to a given specific growth rate value at 1.0 day^{-1} decreased as the temperature increased up to 35°C, indicating an increase of the efficiency of nitrogen uptake with temperature. At 39.2°C the values of S_{N_1} corresponding to a given μ_n increased again, indicating that the optimum temperature with respect to growth.

As indicated in Table III and Figures 9, 11, and 12, the mixed

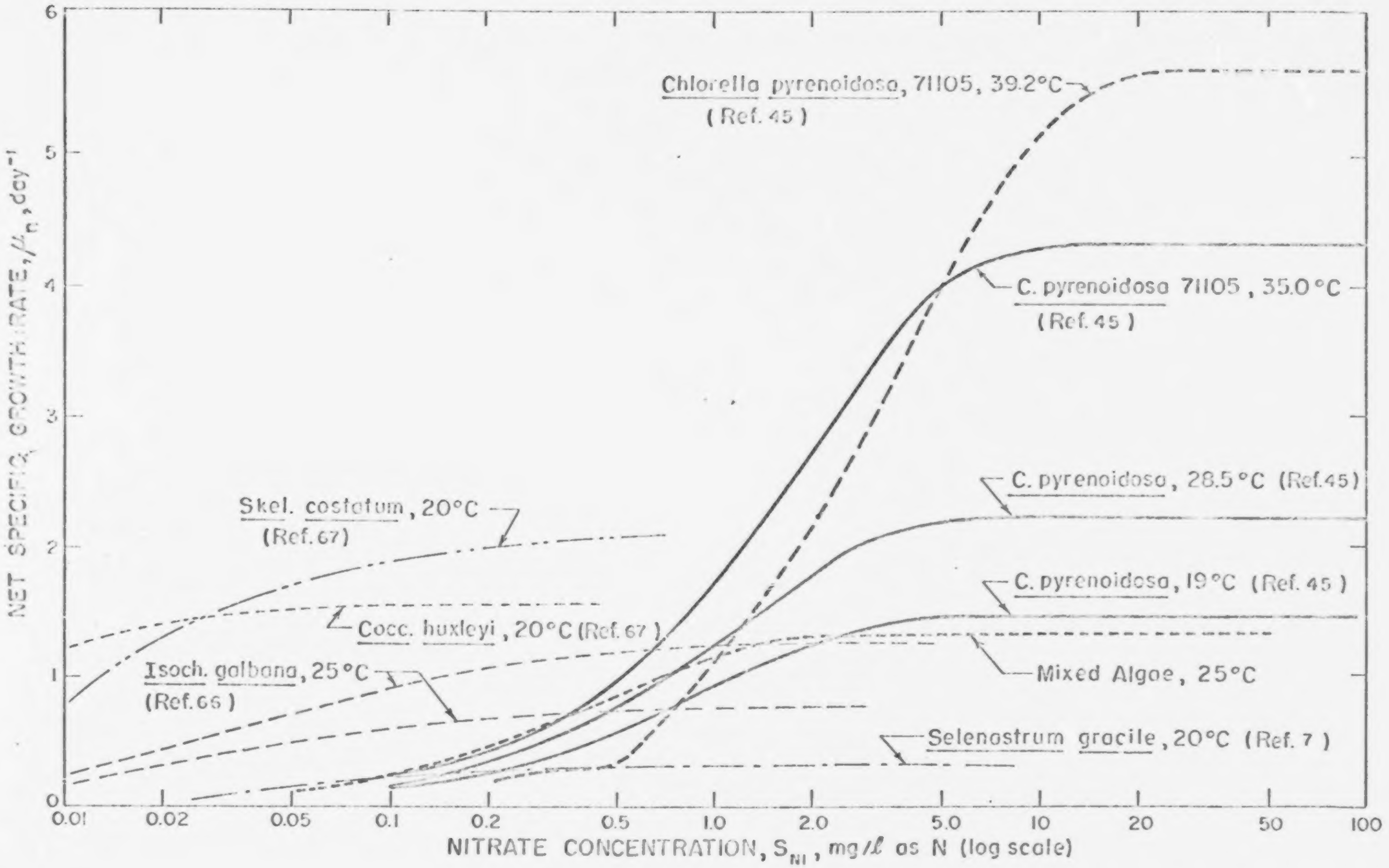

FIGURE 11. A COMPARISON BETWEEN VARIOUS ALGAL POPULATIONS WITH RESPECT TO NET SPECIFIC GROWTH RATE AS A FUNCTION OF NITRATE-NITROGEN CONCENTRATION

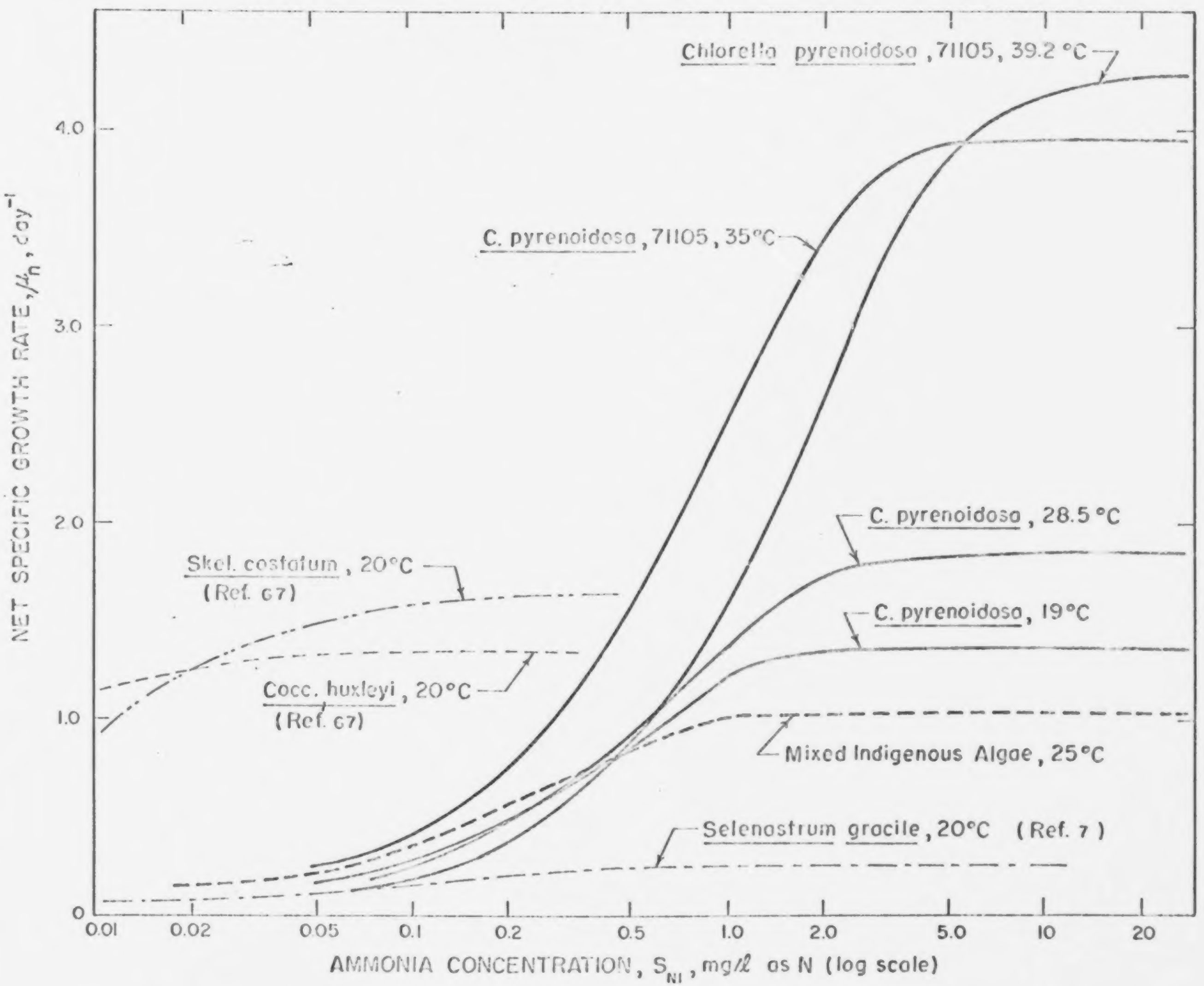

FIGURE 12. A COMPARISON BETWEEN VARIOUS ALGAL POPULATIONS WITH RESPECT TO NET SPECIFIC GROWTH RATE AS A FUNCTION OF AMMONIA-NITROGEN CONCENTRATION

culture of algae indigenous to San Joaquin Delta showed substantial specific growth rate at much lower nitrogen concentrations than *C. pyrenoidosa*.

The low levels of the parameters K_A or K_S are an indication of the good adaptation of the mixed indigenous algae to conditions of lower nitrogen concentrations. The poor adaptation of *C. pyrenoidosa* to low nitrogen concentrations is not unexpected. In fact, the original isolation of *C. pyrenoidosa* was from moist rich soils apparently having a high nitrogen content, while the inocula used in the experiment were obtained from stock cultures maintained on nitrogen-rich media over a number of years. Thus, the selection of *C. pyrenoidosa* as a test organism for algal assaying in natural bodies of water not heavily polluted is rather limited.

The ability of certain species of algae to grow even under extremely low nitrogen concentrations while other species are not adapted to grow under such conditions is evident from Figures 11 and 12. Here data compiled by Caperon (66) of the marine armored flagellate *Isochrysis galbana*, together with data compiled on other marine phytoplankton by Eppley *et al* (67) and data reported in experiments with *Selenastrum gracile* (7, 8) are compared with the data gathered in Experiments 1 through 5 with nitrate (45) and in Experiments 1 through 5 with ammonia in this study. A substantial growth rate of approximately 0.5 day took place with *I. galbana* at at nitrogen concentration as low as 0.02 mg/l (20 μ gm/l) for example.

SUMMARY

The data pertaining to specific growth rate became increasingly consistent with increase in the relative concentration of nitrogen, and at those concentrations permitting full growth, they were quite consistent. Because of the high degree of consistency attained at optimum concentrations of nitrogen, the maximum net specific growth rate, $\hat{\mu}_n$, corresponding to the minimum generation time of the species is an extremely reliable parameter. In the present study, its value was determined prior to initiating each set of experiments. Medium containing nutrient in optimum concentration was used in making the determination, and they were made under identical growth conditions.

In all of the determinations of $\hat{\mu}_n$, a value was found which proved to be constant for a given strain of algae grown in a medium having an optimum concentration of nutrients at a given temperature. Because of the experimentally demonstrated constancy of the value under a defined set of conditions, and its relation to species and temperature, the maximum specific growth rate can serve as convenient and reliable parameter with regard to the genetic characteristics of algae and to physical environmental conditions, especially temperature. Furthermore the value of the parameter $\hat{\mu}_n$ can be drawn directly from the experimental data under optimal conditions and can serve as a parameter in a kinetic model function. This does not hold true with bacterial systems in waste treatment. The observed values for maximum specific growth rates of bacteria in such systems is quite variable, at best they can only be approximated probably because of the mutation rate in bacteria is high compared with that of algae.

The response in the form of increase in growth rate to an increase in the concentration of nitrogen is surprisingly sharp. For example, the gap between a nitrogen concentration supporting a growth rate equal to one-fourth the maximum growth rate and that supporting maximum growth rate can be bridged in most cases by an increase in nitrogen concentration (mg/l as nitrogen) of less than one order of magnitude. This relatively sharp response supports the common assumption of the existence of a "critical concentration" of nutrients, i.e., the concentration at which algal growth is triggered in a natural body of water, and above which even a small increase in concentration results in mass phytoplanktonic production.

The parameters K_A and K_S, which define the concentration of a given growth limiting factor at 0.63 and 0.5 the maximum net specific growth rate respectively for a given algae and a given set of operating parameters, are excellent indicators of how an algal system responds to its environment. For these algal systems that are to be operated near the maximum specific growth rate obtainable, such as waste treatment systems, these parameters describe the steepness of the curves that represent the specific growth rate as a function of concentration of the limiting nutrient. Thus, they give indications of what growth rate will be close to the maximum obtainable. Secondarily, S_{N_1}, the concentration factor corresponding to 0.9 $\hat{\mu}_n$, is a more exact indicator of the concentration of the limiting nutrient required for obtaining the maximum growth rate.

The three parameters, K_A, K_S, and S_{N_1} can be used not only in a mathematical interpretation of the kinetics of the system, but also as guides in determining how close to its maximum capacity an algal system is operating.

III. THE EFFECT ON ALGAL GROWTH OF NUTRIENTS FOUND IN WASTE WATER EFFLUENTS

Conventional waste water treatment systems such as the activated sludge process and the trickling filter are relatively ineffective in removing nutrients (i.e. nitrogen and phosphorus) from waste water. These nutrients are capable of supporting the growth of considerable algal biomass. In addition, the discharge of waste water into receiving bodies of water after varying degrees of treatment which are not designed specifically for nutrient removal can trigger large algal blooms and accelerate problems of eutrophication. It is thus necessary to determine the relative proportion of waste water discharged into a receiving water which still triggers an appreciable algal growth in order to estimate both the dilution capacity of the receiving water and the permissible amounts of nutrients that can be discharged into it.

In order to determine the amount of a particular nutrient that must be removed from a waste water, it is first necessary to determine which of the nutrients constitutes the growth-limiting factor.

An extensive amount of research has been performed on assaying algal growth with respect to nutrient sources. Most of this research, notably that of Skulberg (82), Oswald and Golueke (83), Bartsch (84) and Goldman and Carter (85) has concentrated on the algal growth potential of the receiving bodies of water rather than on the waste water effluents. The nutrients found in waste waters are often major contributors to a receiving body's nutrient load. More recently, however, research has been directed at assaying the algal growth potential of waste water effluents in varying dilutions with the receiving water body (7, 8, 86, 87), since this method appears to have more value in determining the sources of nutrients reaching a receiving water and in planning for their proper degree of removal.

Also, as shown by Gotaas, et al. (16), Oswald and Gotaas (17), Golueke, et al. (7), and others, algal waste treatment methods such as stabilization ponds and aerobic ponds, depend to a great extent on algal biomass production, which is directly related to the rate of photosynthetic oxygenation of the organic matter. Furthermore, growth and production of algal biomass is proportional to the removal of nutrients by assimilative incorporation into algal cellular material. Therefore the elucidation of the relationship between the various nutrients found in waste water effluents and the algal growth potential of the effluent is necessary in order to optimize the design and evaluate the algal waste treatment processes.

This chapter describes three independent experiments in which various types of waste water effluents, at various dilutions, served as media for two species of algal, namely Selenastrum gracile and Chlorella pyrenoidosa. Both batch and continuous culture techniques were employed and a special emphasis was placed on discerning those nutrients which constitute the growth-limiting factors for algal growth.

All three experiments were performed at the Sanitary Engineering Research Laboratory (SERL) of the University of California, Berkeley.

MATERIALS AND METHODS

Experiment 1

Cultures of Selenastrum gracile were inoculated into multiple series of 250 ml flasks with 150 ml of waste water effluents of different degrees of treatment and at various dilutions with distilled water. As was shown in experiments at Lake Tahoe (7, 8), Selenastrum gracile constitutes a proper indicator organism for assay purposes because of its following characteristics:

1) Broad nutrient response;
2) Adaptation to laboratory conditions;
3) Distinctive shape, uniform size and clear division which facilitate quantitative evaluation;
4) Non-attachment to glass;
5) Ubiquity in natural bodies of water.

The initial concentration of algae was approximately 50 cells/mm^3 (50,000/ml) and the carry-over of nutrients by the inoculum was minimized by careful washing and resuspendion of the algae.

Four types of waste waters were tested:

1) Raw sewage from a typical residential area.
2) Primary effluent after approximately 2 hours of primary sedimentation in a settling tank.
3) Secondary effluent from an activated sludge plant (standard rate).
4) Alum treated secondary effluent, in which a 25 mg/l dose of alum was employed for the coagulation and flocculation of the effluent.

All the waste water effluents were obtained from the experimental sewage treatment plant at the Richmond Field Station of the University of California at Berkeley.

The nitrogen and phosphorus content of the various waste waters is given in Table IV.

The first three effluents listed in the table were made up in three dilutions: 0.1%, 1%, and 10%; the alum treated effluent was diluted at 1%, 10% and 50%.

The dilution water was enriched with mineral nutrients excluding

TABLE IV. NITROGEN AND PHOSPHORUS CONTENT OF VARIOUS WASTE WATER EFFLUENTS USED IN EXPERIMENT 1.

Type of Effluent	Concentration, mg/l	
	Nitrogen, Total	Phosphorus
Raw Sewage	29.7	14.6
Primary Effluent	31.4	9.6
Secondary Effluent	23.4	9.0
Alum Treated Secondary Effluent	22.8	6.0

nitrogen and phosphorus as described in Table V and Table VI in order to insure that no other factor other than these two nutrients would limit algal growth.

Three experimental runs were performed, each with 72 flasks. The flasks were shaken continuously at about 100 oscillations per minute and were continuously illuminated with fluorescent light at light intensity levels of 350-400 foot-candles. Some of the control flasks contained dilution water alone while others contained the waste water effluent, dilution water and inoculum but were darkened to prevent any photosynthetic growth.

Algal growth and production were measured by cell count, total suspended solids and volatile suspended solids determinations (88) every two days (up to six days after the inoculation).

Experiment 2

Cultures of *Chlorella pyrenoidosa* (thermotolerant strain TX 71105) were grown in six growth units described by Oswald (70). Each unit consisted of a one-liter cylindrical pyrex culture tube equipped with ports for the injection of a CO_2 - air mixture and each illuminated with four 30-watt "Optima" fluorescent lamps. Light intensity incident on the culture surface was approximately 1000 foot-candles while the temperature of the culture was maintained at 35°C (± 1°C).

Activated sludge effluent obtained from a pilot plant at the Richmond Field Station of the University of California served as the basic medium. The composition of the activated sludge effluent is given in Table VI.

The activated sludge effluent, used as the basic medium in the experiments, was enriched with various nutrients to determine which nutrient could be responsible for accelerating algal growth. The composition of the various media thus formed are given in Table VII. Compounds used in the

TABLE V. COMPOSITION OF NUTRIENT COMPOUNDS IN DILUTION WATER

Compound	Concentration mg/1
$MgSO_4$	18
$CaCl_2$	6
Fe (EDTA)	0.05
NaCl	6
KCl	6
Trace elements solution*	1 ml/1

*See Table VI for composition of trace-element solution.

TABLE VI. COMPOSITION OF TRACE-ELEMENTS SOLUTION (75)

Compound	Concentration mg/1
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
MoO_3	0.015
$CoCl_2 \cdot 6H_2O$	0.04

TABLE VII. COMPOSITION OF ACTIVATED SLUDGE EFFLUENT

Characteristic	Range	Average
Total Suspended Solids, mg/l	5-35	15
Volatile Suspended Solids, mg/l	5-30	12
BOD (5-day, 20°C) mg/l	10-25	20
COD, mg/l	20-40	35
Total Hardness, mg/l as $CaCO_3$	110-170	156
Total Alkalinity, mg/l as $CaCO_3$	60-120	100
pH	7.0-7.7	7.5
NH_3-N, mg/l		0.5
NO_3-N, mg/l	15-25	20
Total Phosphorus, mg/l	8-11	9
Organic P, mg/l	0	0
Inorganic P, mg/l	8-11	9
LAS*, mg/l		0.5
Chlorides, mg/l	65-110	80
Sulfate, mg/l	90-100	92

*Linear Alkyl Sulfonate

preparation of the media were identical with those used routinely at the Sanitary Engineering Research Laboratory (SERL) in the preparation of urea medium as given in Tables VIII and IX. SERL Urea Medium (at half strength) constituted the experimental control. All media were filtered twice through glass wool before use. After inoculation the culture was maintained under continuous illumination until two days after the attainment of maximum algal concentration. Algal biomass was measured by volatile suspended solids.

Experiment 3.

Cultures of Chlorella pyrenoidosa were grown under steady-state conditions in a 9.7 liter chemostatic, spin-type growth unit (the "Algatron"). A detailed description of the "Algatron" growth unit is given by Oswald et al. (89), Shelef, et al. (45), and in Chapter IV of this report. The temperature of the culture was maintained at 27°C (± 1°C), and air + CO_2 mixture was added continuously.

The following light sources were used in this experiment:

a) Two 500 W Iodine-Quartz lamps enclosed in a water jacket which provided an irradiance of 0.171 cal/cm^2-min incident on the culture surface at the center of the culture drum.

b) One 400 W sodium vapor arc lamp (Lucalox) that provided an irradiance of 0.290 cal/cm^2-min incident of the culture surface at the center of the culture drum.

The following media were used in the experiment:

1) Settled domestic sewage from Richmond, California. The composition of the settled sewage used in the experiment is given in Table X.

2) Nitrogen enriched settled sewage in which urea was added to form a 0.025 M concentration.

3) SERL Urea Medium (Tables VIII and IX) served as control medium.

TABLE VIII

COMPOSITION OF S.E.R.L. UREA MEDIUM

Nutrient	Molar Concentration in Media moles/liter
Urea	2.5×10^{-2}
KH_2PO_4	0.6×10^{-2}
K_2HPO_4	0.3×10^{-2}
$MgSO_4 \cdot 7H_2O$	1.0×10^{-2}
Fe Versenol 120	1.74×10^{-4}
Micronutrients	(see Table VII)
$CaCl_2$	1.0×10^{-4}

TABLE IX

MODIFIED ARNON'S MICRONUTRIENT FORMULA

Nutrient	Molar Concentration in Media moles/liter
H_3BO_3	4.6×10^{-5}
$MnCl_2 \cdot 4H_2O$	9.2×10^{-7}
$ZnSO_4 \cdot 7H_2O$	7.7×10^{-7}
$CuSO_4 \cdot 5H_2O$	3.2×10^{-7}
MoO_3 (99.5%)	1.0×10^{-7}
$CoCl_2 \cdot 6H_2O$	1.7×10^{-7}

TABLE X

COMPOSITION OF SETTLED SEWAGE USED IN THE EXPERIMENT

Characteristics	Range	Average
Total Solids, mg/1	804 - 1306	1116
Total Suspended Solids, mg/1	81 - 121	112
Volatile Suspended Solids, mg/1	44 - 82	71
BOD (5 days), mg/1	115 - 175	123
COD, mg/1	225 - 312	258
Total Hardness, mg/1 as $CaCO_3$	125 - 202	156
Total Alkalinity, mg/1 as $CaCO_3$	145 - 212	167
pH	6.8 - 7.6	7.3
Total Nitrogen, mg/1	37 - 45	41
Organic-N mg/1	8 - 25	16
NH_3-N mg/1	14 - 26	25
NO_2-N mg/1	0 - 0.6	0.2
NO_3-N mg/1	0.4 - 3.4	1.7
Total Phosphorus, mg/1	10 - 14	11.0
Organic P mg/1	0.3 - 1.0	0.5
Inorganic P mg/1	9 - 13.0	10.5
LAS* mg/1	4.0 - 11.0	7.0
Chlorides mg/1	65 - 110	80
Sulfate mg/1	90 - 100	92

*Linear Alkyl Sulfonate

Various dilution rate levels were maintained in the culture unit and the algal concentration and algal biomass production were allowed to reach steady-state conditions.

The concentration of the algal biomass in the "Algatron" reactor as well as the daily algal biomass production was measured by the total suspended solids after substracting the bacterial biomass which was calculated using parameters of bacterial growth kinetics and BOD removal as reported by Echoff and Jenkins (90).

RESULTS AND DISCUSSION

Experiment 1

The production of algal biomass grown on various waste water effluents, diluted to 10 percent with distilled water, is given in Figure 13. In this figure there is shown a marked reduction in algal production after six days of growth for secondary and alum treated effluents as compared to raw waste water and primary treated effluents. Several possible explanations to describe this phenomenon are:

a) Algal growth was related to the level of total nitrogen in solution (See Table IV). Since the raw waste water and primary effluent contained more nitrogen than the secondary and alum treated effluents, a higher level of algal biomass was supported with these effluents.

b) Although not shown in Table IV, secondary conventional activated sludge effluents contain nitrogen in the oxidized form of nitrate-nitrogen, while raw waste water and primary effluents contain it in the reduced forms of organic-nitrogen and ammonia-nitrogen. Because more energy is required for algae to assimilate nitrate-nitrogen as compared to ammonia nitrogen, algae utilise the reduced forms of nitrogen most readily.

c) The secondary and alum treated effluents may have been lacking in

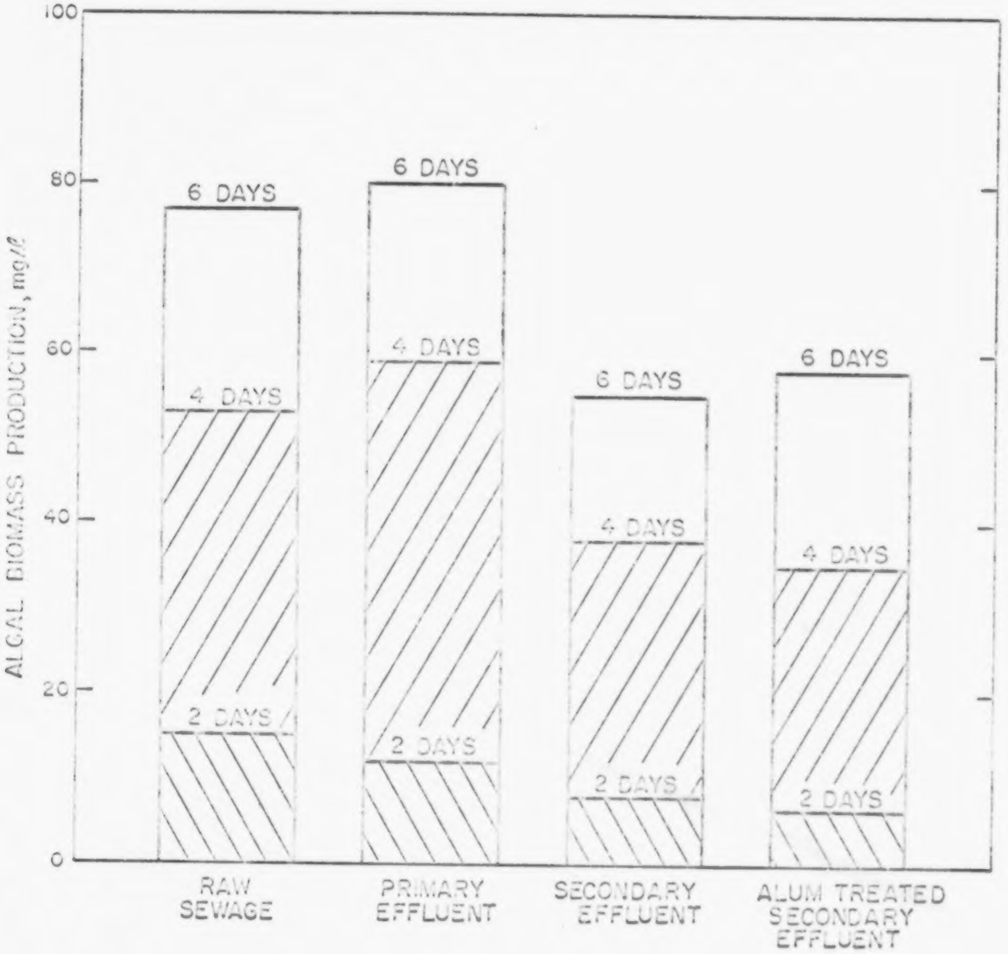

FIGURE 13. ALGAL BIOMASS PRODUCTION (SELENASTRUM GRACILE) IN BATCH CULTURES AS A FUNCTION OF TIME IN WHICH DILUTIONS OF VARIOUS WASTEWATER EFFLUENTS SERVED AS THE GROWTH MEDIUM (10% WASTEWATER, 90% DISTILLED WATER)

certain necessary nutrients such as iron, manganese and/or other trace elements easily precipitated out of solution or absorbed on settleable material. Precipitation could have resulted from the high oxygen levels found in the aeration tanks which oxidized these heavy metals to insoluble forms.

An interesting point concerning the effect of phosphorus on algal growth is that, although there is a considerable difference in phosphorus levels for the secondary and alum treated effluents, there was very little difference in algal growth with these two effluents. This would point to the fact that phosphorus was not a growth-limiting factor in this case.

The increase in algal concentration for the six-day period of the experiment (in two-day intervals) for each type of effluent, at their respective dilutions, is shown in Figure 14. Both the 0.1% and the 1% dilutions supported a minimum algal population regardless of the type of effluent used. This was undoubtedly due to the fact that the nitrogen level in both cases was extremely low. For the 0.1% dilution it was less than 0.03 mg/l and for the 1.0% dilution it was less than 0.3 mg/l.

The maximum growth rate for the 10% dilutions occurred between the second and the fourth day after the test began and then proceeded to level off. In the 50% dilution for the alum treated water, however, the maximum rate of growth occurrred between the fourth and sixth day.

Thus, the effect of initial nutrient concentration on the peaking-out time for algal growth is an important consideration when attempting to develop a convenient point at which to terminate an algal bioassay test. It becomes apparent that it is extremely important to carry a test to a point where no further growth is observed for a period of time, say two days. Then, there is an assurance that the full algal growth potential of the water was measured.

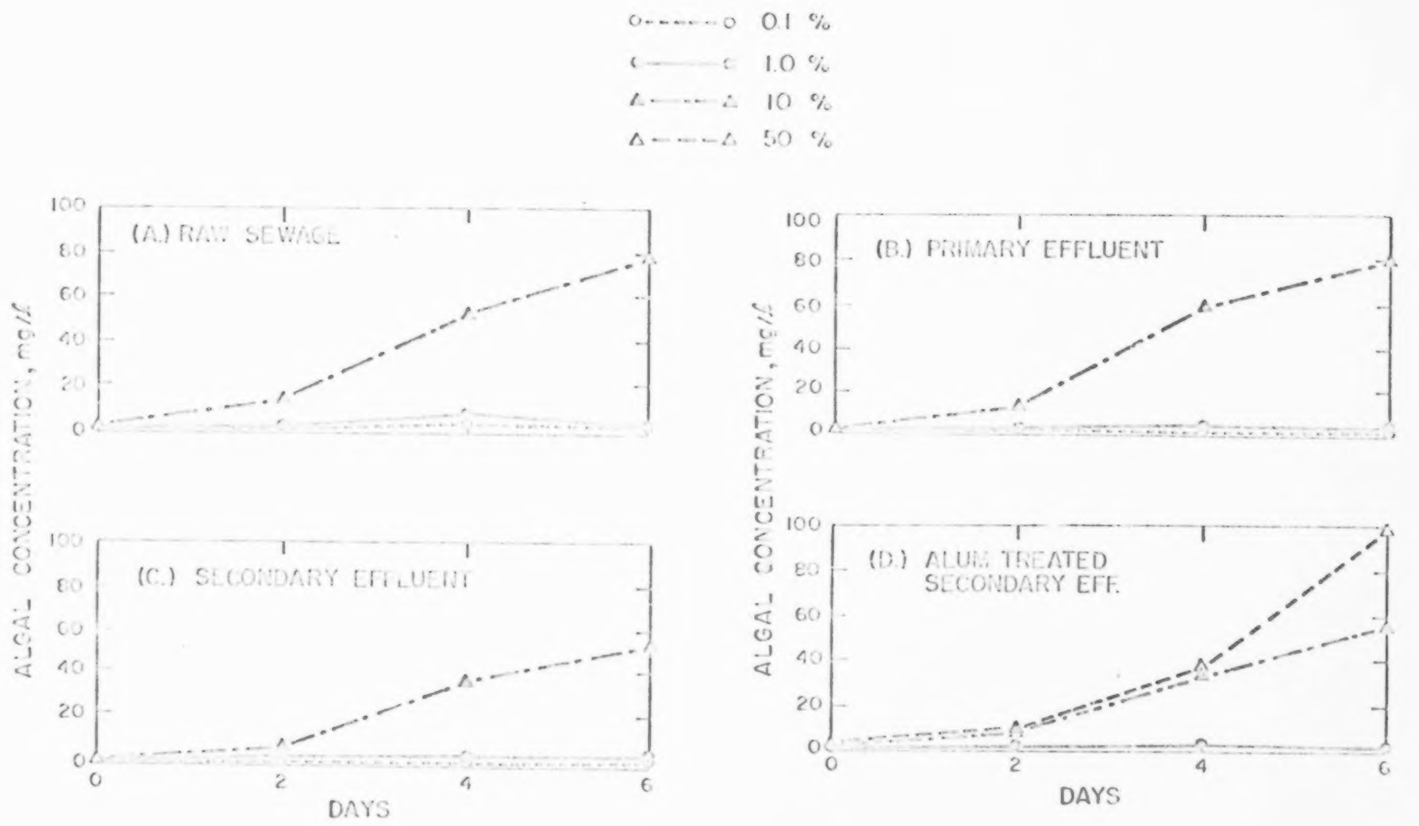

FIGURE 14. ALGAL BIOMASS PRODUCTION (SELENASTRUM GRACILE) IN BATCH CULTURES AS A FUNCTION OF TIME IN WHICH DILUTIONS OF VARIOUS WASTEWATER EFFLUENTS SERVED AS THE GROWTH MEDIUM

The effect of dilution on the concentration of algae, as expressed by cell-count, is also given in Figure 15. The small algal production which occurred when the dilution of the waste water effluents remained below one percent, gives some indication of the permissible dilution of waste waters in receiving bodies of water with regard to the prevention of algal blooms.

Experiment 2

The maximum concentrations of algal biomass reached with activated sludge effluent and its various enrichments as compared with SERL Inorganic Medium is given in Figure 16.

The addition of nitrogen in the form of urea increased the maximum concentration more than two-fold. The addition of micronutrients (the composition of trace elements is specified in Table IX) caused a further increase. Iron also caused a significant increase. The addition of both iron and micronutrients brought the maximum concentration to a level which is two times the concentration of activated sludge enriched with nitrogen only.

The synergistic effect observed by the addition of both iron and the micronutrients solution might be explained by the role of iron and the other trace elements, notably manganese in the production and formation of chlorophyll (91, 92).

The maximum concentration of algae when activated sludge effluent enriched with nitrogen, iron and micronutrients served as the growth media, was approximately the same as when inorganic media were used. Thus, it was clear that phosphorus did not constitute a growth-limiting factor with the given levels of light intensity. The fact that nitrogen, iron and micronutrient levels in waste water effluents constitute growth limiting factors while phosphorus is relatively in excess, is important in dealing with eutrophication trends caused by waste waters discharged into receiving bodies

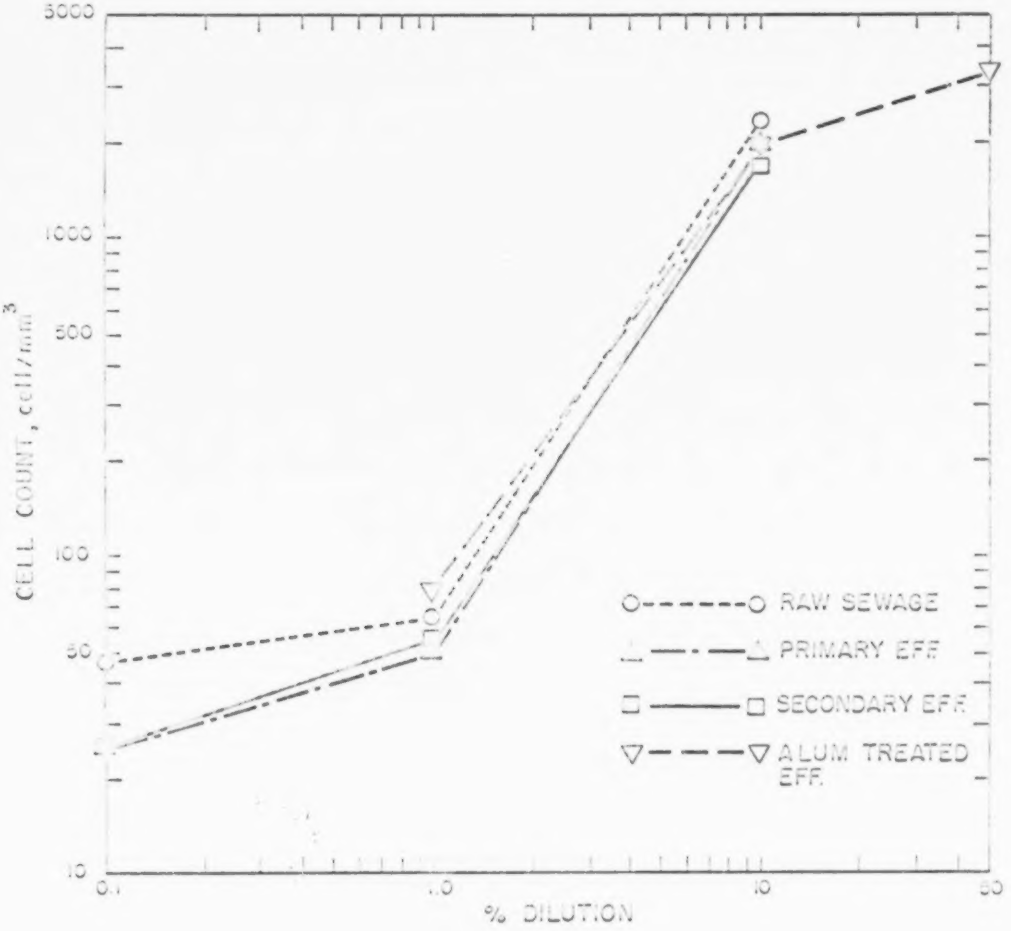

FIGURE 15. ALGAL BIOMASS PRODUCTION (*SELENASTRUM GRACILE*) AFTER 6 DAYS IN BATCH CULTURES AS A FUNCTION OF DILUTION IN WHICH VARIOUS WASTEWATER EFFLUENTS SERVED AS THE GROWTH MEDIUM

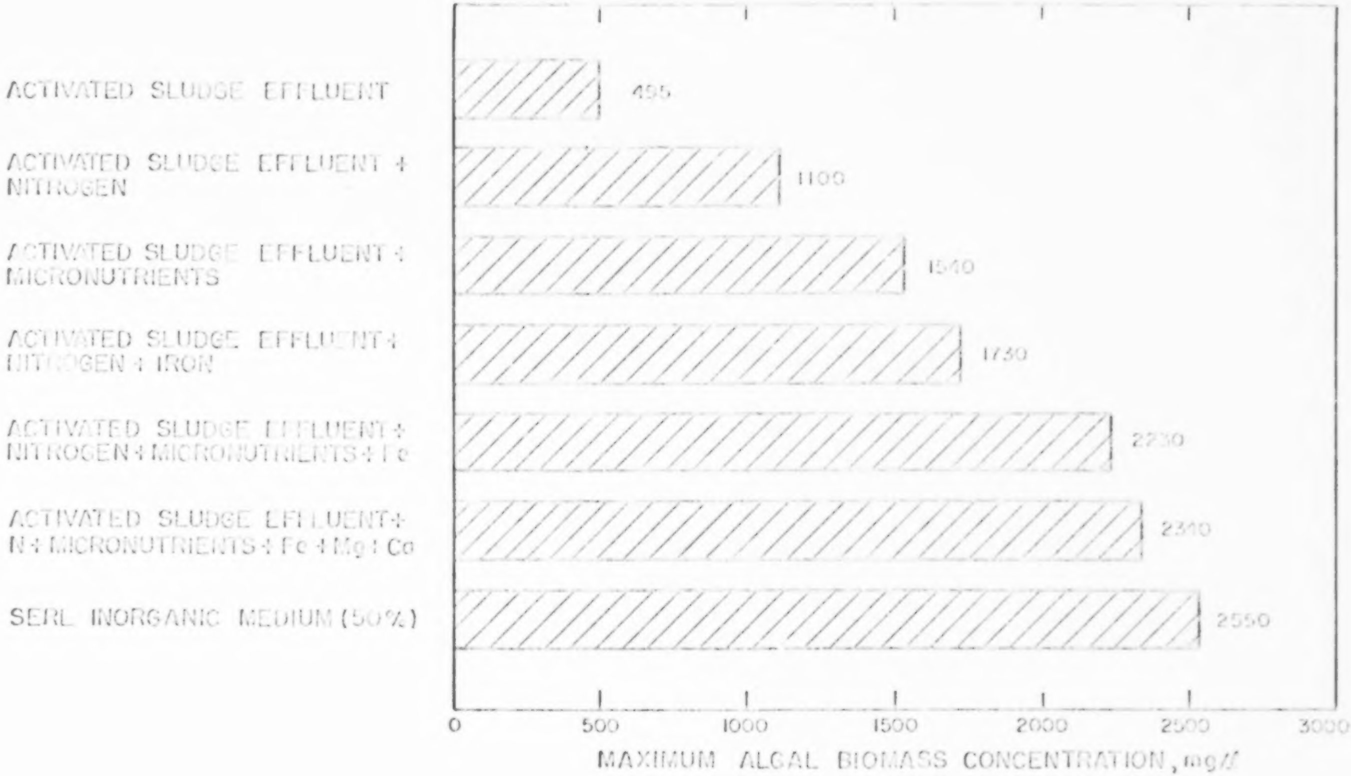

FIGURE 16. MAXIMUM ALGAL BIOMASS CONCENTRATION (CHLORELLA PYRENOIDOSA) IN CONTINUOUS CULTURE AS A FUNCTION OF NUTRIENT ENRICHMENT TO ACTIVATED SLUDGE PLANT FINAL EFFLUENT GROWTH MEDIUM

of water and for sanitary engineering considerations of nutrient removal. However, it should be pointed out while phosphorus may not be the growth limiting factor in waste water effluents, receiving waters may still contain enough phosphorus from other sources so that it may still act to stimulate enought algal growth so as to cause nuisance conditions.

Experiment 3.

The relationship between the applied dilution rate and algal biomass production when settled sewage, N-enriched settled sewage and inorganic medium were used are given in Figure 17 for the Iodine-Quartz light source and in Figure 18 for the "Lucalox" light source.

The effect of the applied dilution rate on the resulting algal concentration in the reactor for the various media and for the aforementioned light source is given in Figures 19 and 20.

It should be emphasized that experiments with continuous cultures can be most practical when considering bodies of water with low residence-times. Thus, the parameter of applied dilution rate and its effect on algal production and concentration can be of great value or almost useless depending on the body of water to which it is applied.

From the experimental results with both light sources, it appears that for low and intermediate levels of dilution rates, enrichment with nitrogen resulted in a significant increase in algal production and concentration. With high dilution rates which in general are accompanied by relatively low algal concentrations, the algal production rates were almost independent of the degree of enrichment of the media, and light probably becomes the sole growth-limiting factor.

Comparing the various media with respect to their ability to support algal production and concentration, it is clear that enrichment with nitrogen

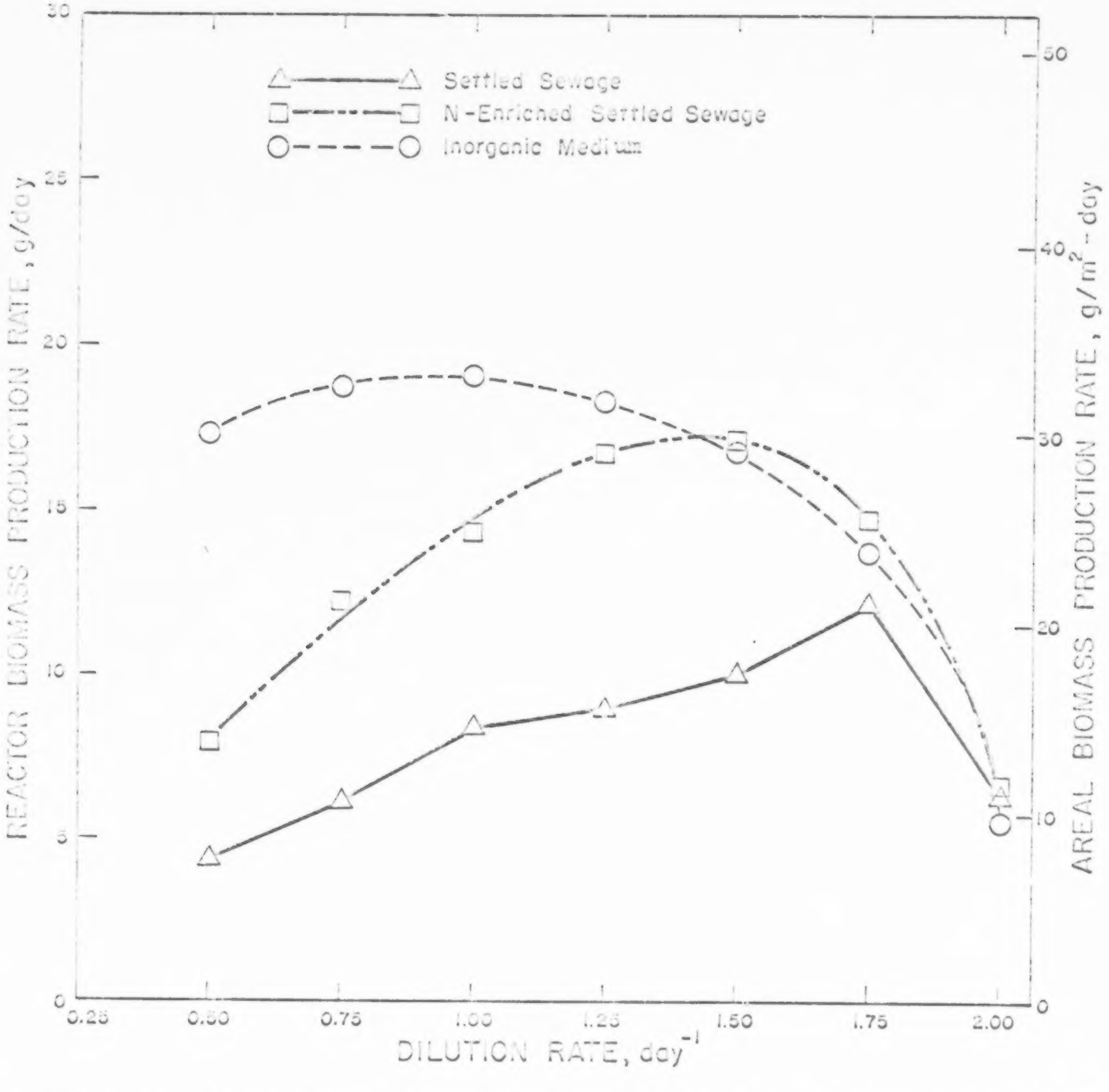

FIGURE 17. Algal Biomass Production Rates (Total and Areal) as A Function of Applied Dilution Rate in Experiments in which Settled Sewage Served as the Medium and Iodine-Quartz Lamps as the Major Light Source.

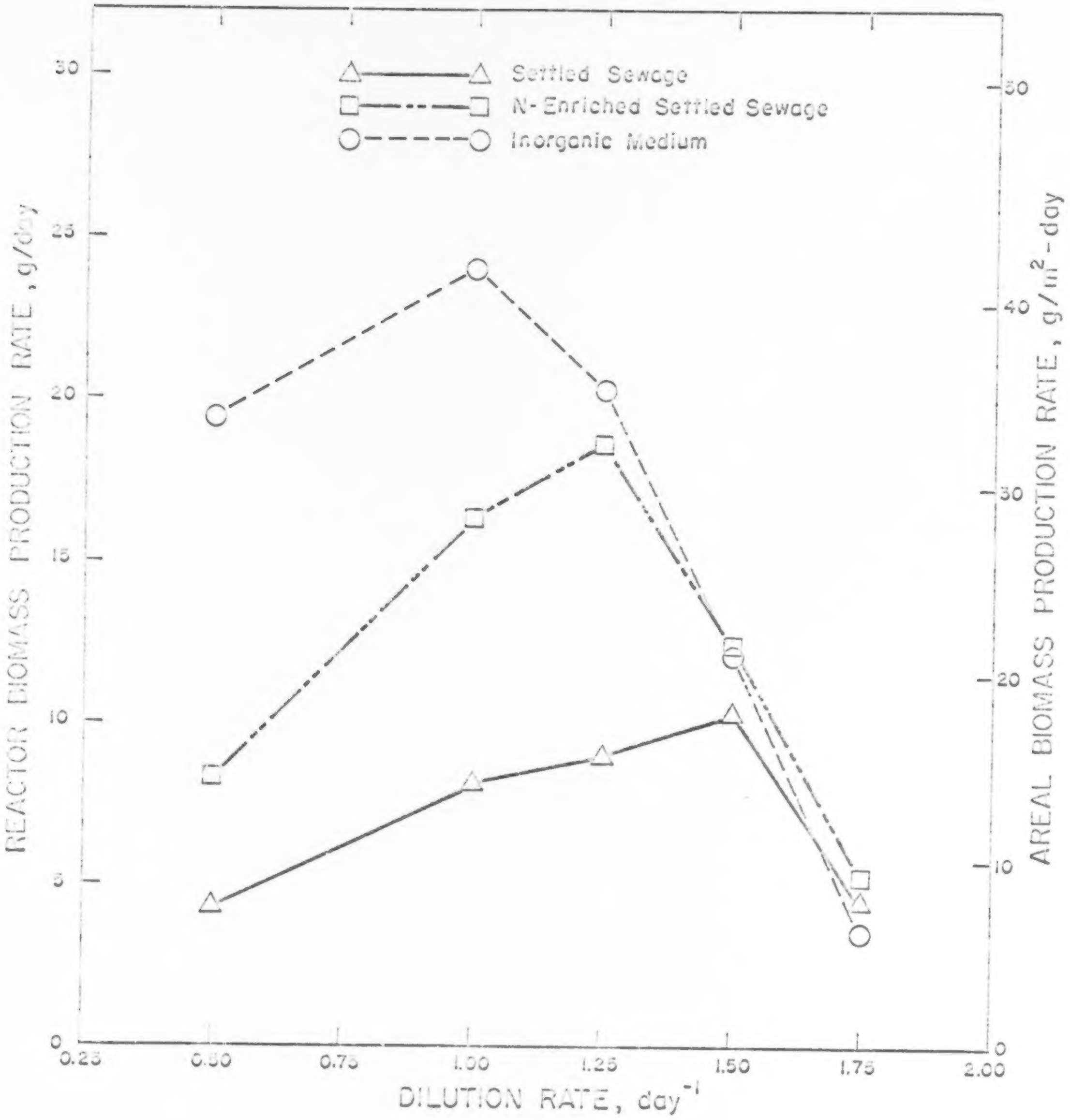

FIGURE 18. Algal Biomass Production Rates (Total and Areal) as a Function of Applied Dilution Rate in Experiments in which Sewage Served as the Medium and a *Lucalox* Lamp as the Light Source.

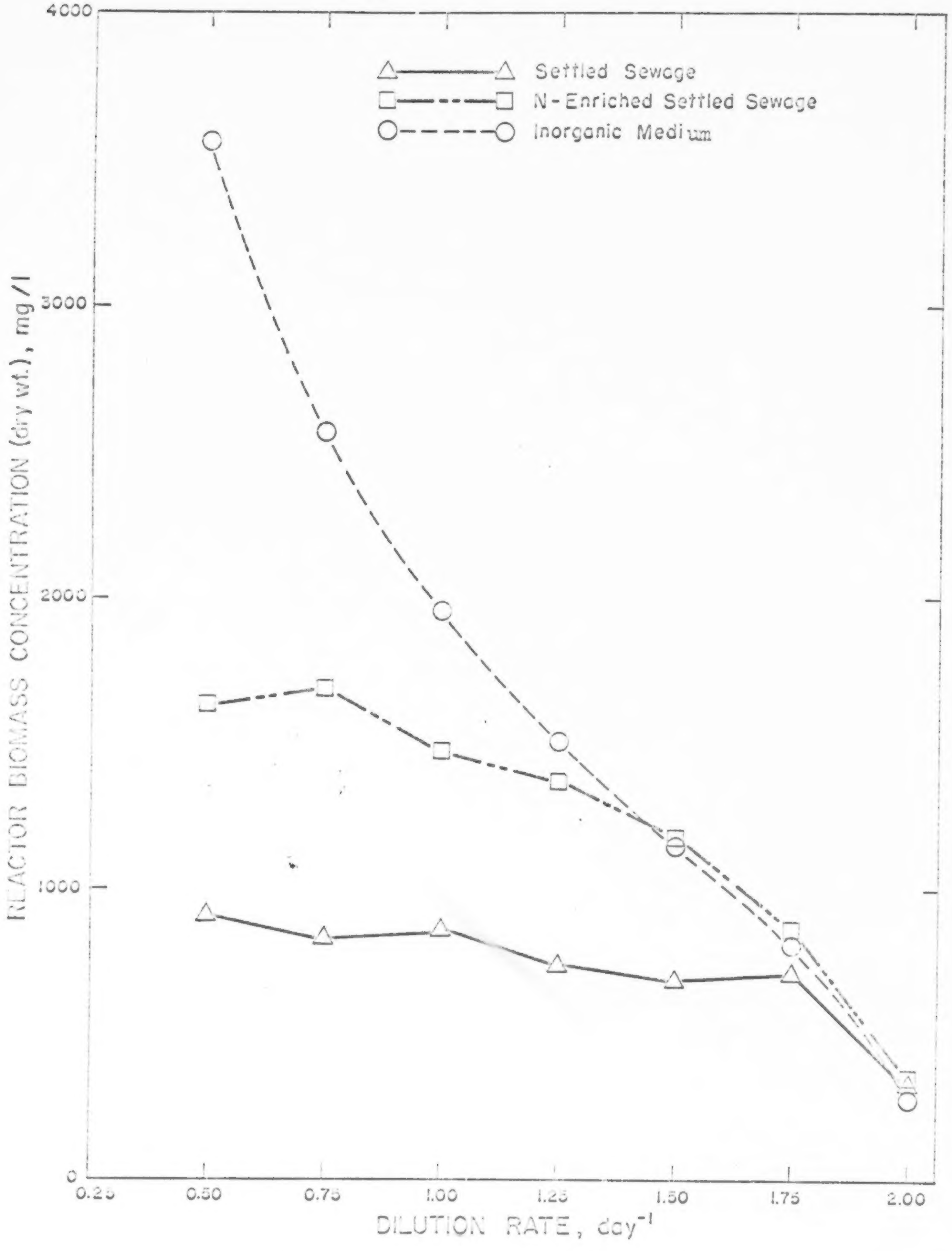

FIGURE 19. Algal Biomass Concentration as a Function of Applied Dilution Rate in Experiments in which Sewage Served as the Medium, and Iodine-Quartz Lamps as the Major Light Source.

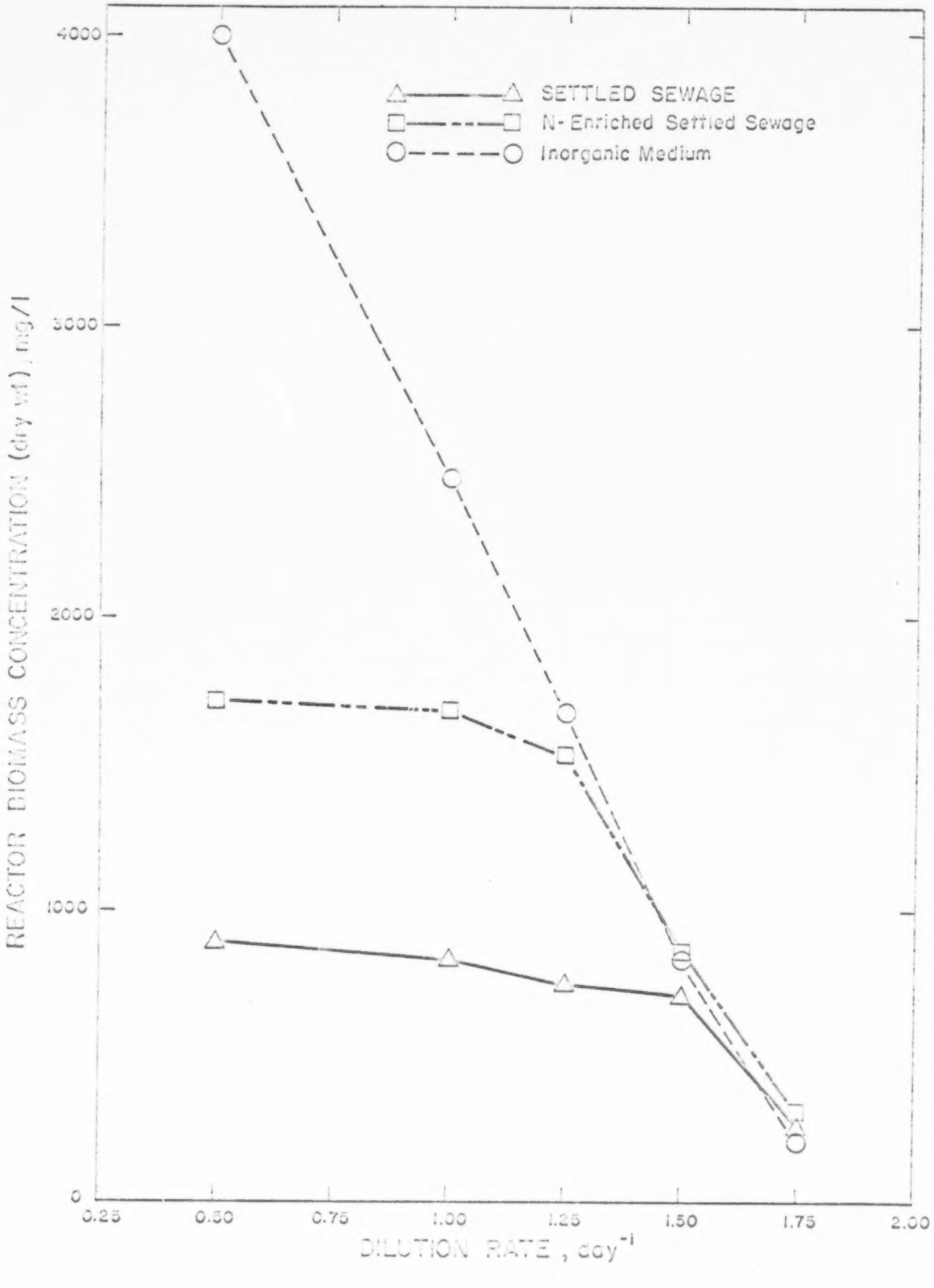

FIGURE 20. Algal Biomass Concentration as a Function of Applied Dilution Rate in which Sewage Served as the Medium and a *Lucalox* Lamp as the Major Light Source.

alone, although increasing the production, was not enough to bring the production levels to those accomplished with the inorganic medium. This is in line with the conclusions drawn in Experiment 2 where activated sludge effluent constituted the basic medium.

Algal production for the various media reached maximum values in a range of dilution rates of between 0.8 to 1.2 day^{-1}. In the very low dilution rates (i.e. 0.5 day^{-1}) the enrichment of nitrogen was less marked; this might be due to the relatively high concentration of algae existing in the reactor under low dilution rates where the effects of crowding interfered in reducing the algal production accompanied by the effect of nitrogen starvation.

CONCLUSIONS

From the results of these experiments it can be concluded that with the waste water effluents tested, dilutions greater than 1 percent had a significant effect on algal production. Thus, it appears that it is possible to use the bioassay method to determine the assimilative capacity of a receiving body of water. The method appears to be a promising way to determine in the laboratory nutrient responses at varying concentrations as might occur in a natural receiving body. From information obtained in this experiment, permissible dilution ratios for waste water discharges into receiving bodies can be formulated.

Similarly the need to carry these bioassays past the point of peak production was demonstrated, since initial nutrient level determines the time for peaking-out.

Nitrogen appeared to be the most significant growth-limiting macronutrient in these experiments while phosphorus was in considerable excess. Iron also appeared to play a significant role as a growth-limiting nutrient.

Similarly, the applied dilution rate had a marked effect on determining the role in which nutrients of a given concentration act as growth-limiting factors. At high dilution rates (short detention periods) the effect of a given nutrient level is much less pronounced than at a low dilution rate (long detention period). The application of this phenomenon to studying eutrophication problems in natural water systems is obvious. For bodies of water such as streams, tidal estuaries and other water systems with short retention times higher nutrient levels may be permissible than in long retention systems such as lakes and large, slow-moving rivers.

Thus, the use of the bioassay technique, both in batch and continuous culture, can be an important tool in trying to assess the effect of a waste water discharge on a receiving body of water.

IV. MAXIMUM CAPACITY AND MODIFIED DESIGN OF THE ALGATRON REACTOR*

INTRODUCTION

The Algatron is a spin-type photosynthetic reactor developed by Oswald _et al._ which has been fully described in the literature (89, 93, 94).

This unit was used for the basic study on the kinetics of algal systems with respect to light as described in the first report of this study (45).

A schematic diagram showing the detailed arrangement of the unit is given in Figure 21. A photograph giving a general view of the unit is shown in Figure 22. In Figure 23 are shown the position of one of the types of the light sources and the internal arrangement of the unit. Dimensions pertinent to the unit are listed in Table XI.

The Algatron was operated under chemostatic conditions which were maintained by injecting a measured amount of medium at preset intervals. The injection periods were programmed for periods ranging from 15 to 35 minutes, and by simultaneously displacing an equal amount. Components of the measuring and injection systems are indicated by numbers 1, 2, 3, 6 and 7 in Figure 21. Displacement was by way of a scoop (17 in Fig. 21) positioned over the rotating film in such a manner that when the depth of the film exceeded a preset level, the excess was scooped off. The unit was not equipped with a thermostatic temperature control. Temperature was maintained at the desired

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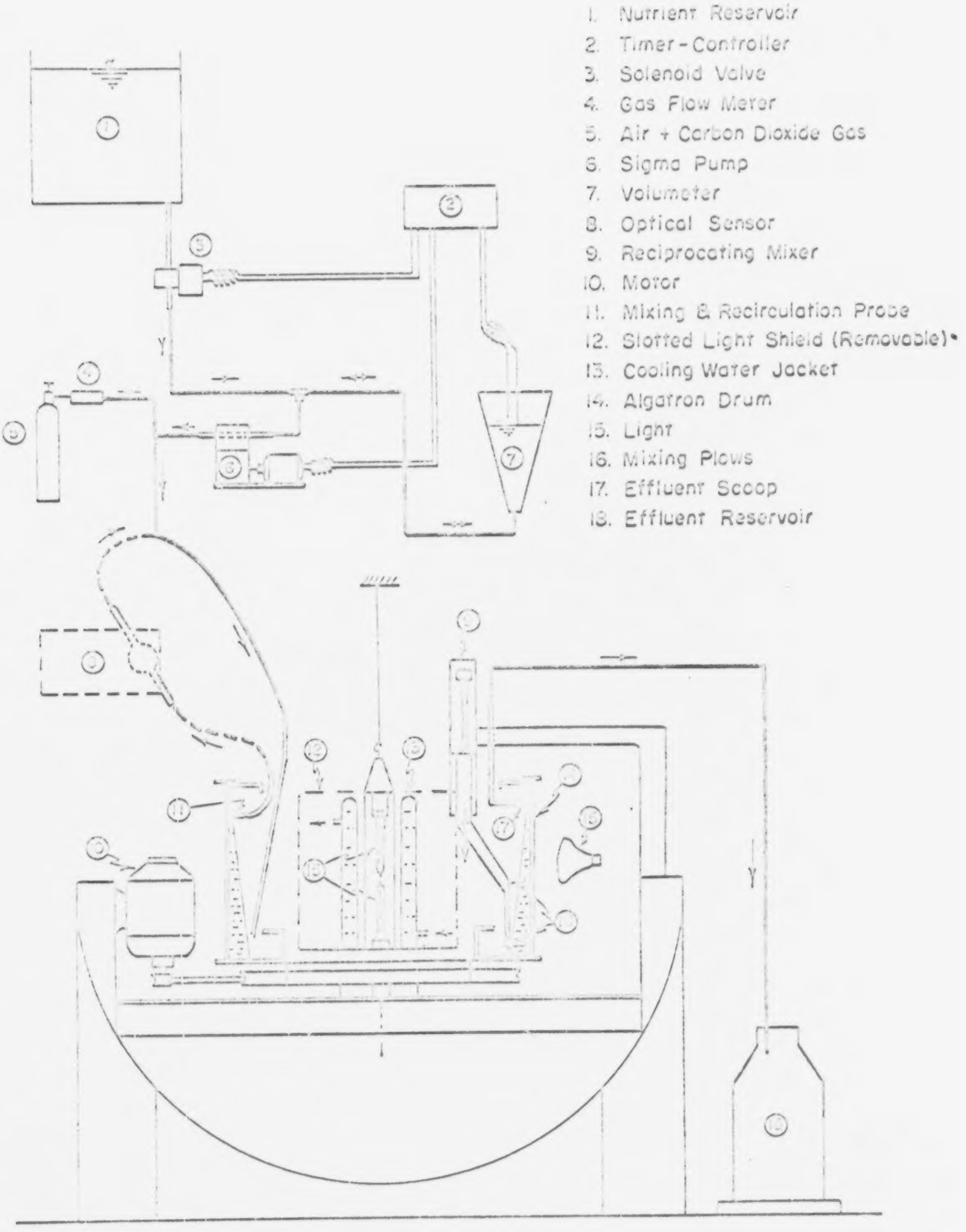
*The reader is referred to Chapters II, III, VI and VII of Reference (45) for a complete description of the Algatron as it was used in the studies comprising the work of this project.



1. Nutrient Reservoir
2. Timer-Controller
3. Solenoid Valve
4. Gas Flow Meter
5. Air + Carbon Dioxide Gas
6. Sigma Pump
7. Volumeter
8. Optical Sensor
9. Reciprocating Mixer
10. Motor
11. Mixing & Recirculation Probe
12. Slotted Light Shield (Removable)•
13. Cooling Water Jacket
14. Algatron Drum
15. Light
16. Mixing Plows
17. Effluent Scoop
18. Effluent Reservoir

FIGURE 21. Algatron Prototype — Arrangement and Components.

NOT REPRODUCIBLE

Figure 22. Algatron Prototype - General View

NOT REPRODUCIBLE

Figure 23. Algatron Drum (A) With Iodine-Quartz Light Source in Water Jacket (B)

TABLE XI

IMPORTANT DIMENSIONS OF VARIOUS COMPONENTS IN THE ALGATRON PROTOTYPE

Component	Dimensions
Algatron Drum	
Diameter	61 cm
Height	30.5 cm
Circumference	192 cm
Motor, RPM	1725
Drum, RPM	210
Radial Acceleration	15.1 x g
Linear Velocity	672 cm/sec
Culture (film during rotation)	
Height	30.5 cm
Depth - average	1.71 cm
maximum	2.72 cm
minimum	0.7 cm
Surface Slope	1:15.1
Volume	9700 ml
Liquid-Air Surface Area	5750 cm^2
Specific Liquid-Air Surface	0.594 cm^{-1}*

*Specific Liquid-Air Surface = $\frac{\text{Liquid-Air Surface Area}}{\text{Volume}}$

level by means of a combination of infrared lamps and ventilation. Despite the lack of a more sophisticated temperature control system, the culture temperature never varied by more than ± 1.5°C.

RECIPROCATING MIXER

A major modification of the reactor was the installation of a reciprocating mixer designed to serve as a means for preventing algal cells from adhering to the wall of the drum. The degree and time at which the cells begin to accumulate and adhere to the drum wall vary according to culture conditions. Factors contributing to this undesirable development are incident light intensity, pH level, dilution rate, CO_2 concentration, nutrient level, toxic substances in trace amounts (e.g. insecticides), and genetic traits. Aside from the inconvenience arising from the frequent cleaning of the drum wall to keep the culture from failing, the formation of a film, even in its initial stages, leads to a marked drop in overall culture yield and efficiency. These factors soon made it apparent that some type of reciprocating mixer was needed. Consequently, the one diagrammed in Figure 21 (item 14) and pictured in Figure 24 was designed and installed.

In essence, the working portion of the mixer consists of a vertical row of steel strips. The strips are submerged in the rotating culture film. Each strip extends to within 0.5 to 1.0 mm of the interior surface of the drum wall. The strips act as plow blades and scoop the algal suspension in a whirling fashion. A mechanism is provided to continuously move the strips up and down the wall in such a manner as to bring about a complete scanning of the drum wall. Four blades were used in the experiments described herein. More blades could have been attached had a higher degree of mixing been needed. The mechanism for moving the blades is as follows: The blades are mounted on a bracket plate, which in turn is attached to a nut in a vertical

Figure 29. Reciprocating Mixer

position. A small reversible electric motor rotates a vertical screw which imparts a reciprocating motion to the nut. Two limit switches mounted along the screw control the extent of the reciprocating motion. The length of the stroke is regulated by the positioning of the switches. The up-and-down cycle time of the mixer can be set to be completed within an interval of one-half to one minute. The small electric gear motor consumes approximately 14 watts of energy. Motors having rotational speeds of 30 and 60 rpm were used.

ADVANTAGES AND DISADVANTAGES OF ALGATRON USED IN THIS STUDY

The increased illuminated surface, bilateral illumination, provisions for application of diffused light, and the use of a thin layer of dense algal culture were some of the principles used in the design and operation of the Algatron prototype. Other advantages, because of its unique design, include: 1) an intrinsic gravitational field -- an important asset in space applications; 2) a relatively extensive liquid-air interface to provide a high rate of gas exchange; 3) a geometry of the unit which allows the application of high-intensity light sources; 4) a rate of heat loss due to the rotation and design of the drum which is sufficiently high to eliminate the need of a cooling system; 5) a high rate of evaporation which provides an excellent source of low temperature distilled water; 6) a convenient application of diffused light and utilization of the flashing light effect which makes possible high light conversion efficiencies; 7) elimination of foaming and adhesion of algal cells to the growth unit walls; and 8) a minimum of maintenance.

A disadvantage, however, is the power penalty imposed due to the rotation of the drum. This penalty could be reduced by applying a dual power system -- one for bringing the drum from standstill to operational rotational velocity, and the second for maintaining the operational velocity.

THEORETICAL MODELS AS APPLIED TO AN OPTIMIZED ALGATRON

As was mentioned above, the Algatron prototype used in previous experiments had unique geometrical characteristics. A noteworthy example of these characteristics was the slope of the exposed surface culture, viz. 15.1:1.0, which was attained when the drum was in motion. With such a configuration, the culture depth at the bottom rim of the drum was 2.7 cm while at the top rim it was about 0.7 cm. Because of the graduation in depth from upper to lower levels of the culture, at a given algal concentration, the path of light in the reactor varied accordingly by a factor of almost four. Although this factor had to be and was fully accounted for in establishing each of the kinetic models, provision for it does result in some distortion between results expected on the basis of the models and those which would be obtained in experiments in which the depth of the culture was uniform throughout the culture. One manifestation of the distortion would be a reduction in expected net algal production as compared to the production in a reactor of optimum design of equal volume and surface area, but in which depth is uniform throughout. For example, under conditions such that 99% of the light is absorbed by the culture at the lower lip of the drum, only 70% would be absorbed in the portion at the upper lip of the drum. In such a situation most of the light is absorbed at the base of the culture, but as the depth of the culture becomes less because of the graduation in slope of the surface, more light passes through and escapes from the culture, until at its uppermost proportion, 30% of the light is passed through and not used.

In addition to the distortion resulting from the graduation in depth, there is a magnification of the variation normally found in the distribution of irradiance by any artificial light source (cf. Appendix F, Figures 68 and 72 in Reference (45)) which resulted from the shading of that portion of the culture found at the bottom rim of the drum. The shading was a function of the structure of the drum used in the previous studies. The unequal incident irradiation distribution and the existence of shaded portions of culture contributed to the reduction in the potential performance of the current unit.

The kinetic models with respect to light developed in the previous study (45) and tested on the Algatron prototype were applied to a theoretical "ideal" Algatron in order to determine the theoretical full capacity of an Algatron built specifically to maximize algal biomass production.

The following models were used for the theoretical calculations (refer to Chapters II, III and VII of Reference (45).

For Model I

$$P_{nal} = \frac{1440\ E_m}{J\ R_t} I_o \left[\frac{I_s}{I_o} \left(\ln \frac{I_o}{I_s} + 1 \right) \int_{400}^{700} I_{\lambda R}\ d\lambda - \frac{I_s}{I_d} \left(\ln \frac{I_d}{I_s} + 1 \right) \int_{400}^{700} I_{\lambda R} \exp\left(-\alpha_\lambda\ C_c\ d \right) d\lambda \right] - \left[10^{-3}\ K_d\ C_c\ d \right] \qquad (4\text{-}1)$$

(Refer to Glossary for explanation of symbols)

in which P_{nal} is expressed in milligrams of dry algae per cm^2 per day and all irradiance values are expressed in cal/cm^2-min.

The net specific growth rate of the algal biomass $\bar{\mu}_n$ can be written:

$$\bar{\mu}_n = \frac{10^3\ P_{nal}}{C_c\ d} \qquad (4\text{-}2)$$

In a chemostatic continuously stirred tank reactor (CSTR) unit at steady state in which the biomass is not recycled, $\bar{\mu}_n$ is equal to the dilution rate Q/V.

The computerized form of Equation (4-1) is given as follows:

$$P_a(h') = \frac{5.645}{J\ 1/\phi\ R_t} I_o(h') \left[\frac{I_s}{I_o(h')} \left(\ln \frac{I_o(h')}{I_s} + 1 \right) \sum_{400}^{700} Q_\lambda\, \lambda\, I_{\lambda R} \right.$$

$$\left. - \frac{I_s}{I_d(h')} \left(\ln \frac{I_d(h')}{I_s} + 1 \right) \sum_{400}^{700} Q_\lambda\, \lambda\, I_{\lambda R}\, e^{-\alpha_\lambda C_c d(h')} \right] \qquad (4\text{-}3)$$

For Model II:

$$P_{na2} = \frac{1440\ E_m}{J\ R_t} I_s \int_{400}^{700} I_{\lambda R} \left[\ln \frac{I_s + I_o}{I_s + I_o \exp\left(-\alpha_\lambda C_c d\right)} \right] d\lambda - 10^{-3} K_d\, C_c d \qquad (4\text{-}4)$$

P_{na2} is expressed in the same units as P_{na1} in Model I and Equation (4-2) applies almost to Model II by using P_{na2} instead of P_{na1}.

The computerized form of Model II is described in Reference (46) pages 87-88.

For Model III

$$P_{na3} = \frac{1440\ E_m}{J\ R_t} I_s \int_{400}^{700} I_{\lambda R} \left[\alpha_\lambda C_c d + E_i\left(\frac{I_o}{I_s}\right) \right.$$

$$\left. - E_i\left(\frac{I_o}{I_s} e^{-\alpha_\lambda C_c d}\right) \right] d\lambda - 10^{-3} K_d\, C_c\, d \qquad (4\text{-}5)$$

In Equation 4-5 P_{na3} is expressed in units of mg/cm^2-day, I_o and I_s are expressed in cal/cm^2-min. An equation similar to Equation (4-2) applies also to Model III.

The computerized form of Model III is described in Reference 46, pages 87-88.

As a point of interest, the theoretical models were applied in the computation of a relationship that would exist between P_n, P_{nv}, $\frac{Q}{V}$ (or $\bar{P}_n$) C_c, E_c, and other performance parameters for an Algatron of optimum design and identical in overall dimensions and total volume with those of the model used in the experiments. Other assumed conditions were that the depth be uniformly 1.71 cm (depth at h' = 15 cm in a rotating culture), and that the light sources used in the experiments also be used in the computations but with a constant incident irradiance applied to the entire surface of the idealized culture. The incident irradiance normally occurring at that line at which h' = 15 in the experimental cultures was 0.171 cal/cm^2-min with the Iodine-Quartz lamp and 0.290 cal/cm^2-min with the sodium vapor arc (Lucalox) lamp. Therefore, in the computations, the entire surface of the cultures was assumed to be uniformly irradiated at either of these two levels. Since input values characteristic of those found at h' = 15 cm in a rotating culture were used in the computations, outputs also were assumed to be those found at h' = 15 cm in the experiments. Therefore, the total output of the idealized Algatron was assumed to be the product of the output at h' = 15 cm and the total surface area.

Practical applications in which the idealized conditions outlined in the previous paragraph could be provided are an outdoor pond of uniform depth, an Algatron having a horizontal configuration, and an Algatron of either horizontal or vertical configuration functioning in space, i.e. outside the gravitational force of the earth. With a horizontal configuration, it is possible to prevent shading of any region of the culture and to provide greater uniformity with respect to geometrical distribution of light than it is possible with the vertical configuration.

Results obtained with the application of three theoretical models to the idealized situation described in the preceding paragraphs are plotted in Figures 25 through 28. Figures 25 and 26 are based on the use of the Iodine-Quartz lamps as the light source; Figures 27 and 28 on the sodium vapor arc (Lucalox) lamp as the light source.

Judging from the experimental results reported in Reference (45), it might be assumed that Model III would best represent the expected performance of the optimized Algatron; and hence that its applicability would be restricted by the limitations described previously for the model. If the assumption be valid and should it be further assumed that the maximum net production for Model III (P_{n3}) represents the expected maximum net production, an expected significant increase over that which takes place in the actual Algatron would occur in net algal production. This would correspond to an increase in dilution rate and the predicted algal concentration at $\hat{P}_n$ in the optimized Algatron thus turns out to be not much greater than that attainable in the actual Algatron. Of course, the overall light conversion efficiency is lower in the idealized situation because of the higher incident irradiance assumed. As stated previously, the maximum incident irradiance at h' = 15 cm was assumed to apply to the entire surface of the idealized culture.

That the Algatron with the Lucalox lamp as a light source is an excellent research tool for studying the kinetic characteristics of algal systems is proved by the fact that on the basis of predicted values shown to parallel experimental values, the expected $\hat{P}_{nv}$ in an optimized Algatron would equal 3.25 g/1-day; P_{na}, 54.9 g/m^2-day; and E_e at $\hat{P}_n$ 3.29 g/kw-hr. Stepping up the incident irradiance through the use of light more intense than that from the Lucalox lamp should result in even higher values for $\hat{P}_n$, $\hat{P}_{nv}$, $\hat{P}_{na}$, and $\hat{\mu}_n$ and C_c at $\hat{P}_n$. However, the efficiencies E_o and E would decline accordingly.

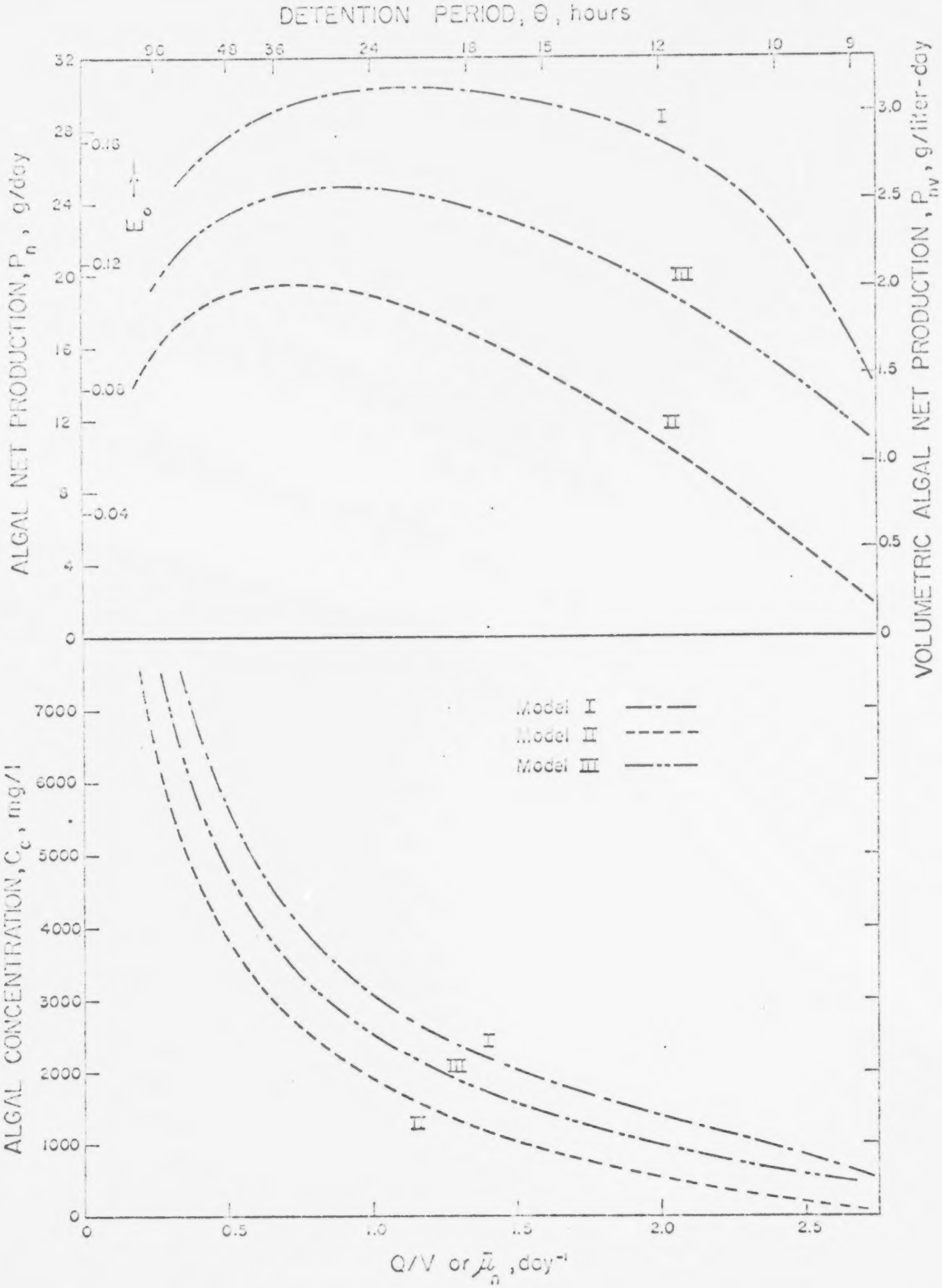


FIGURE 25. THEORETICAL KINETIC MODELS AS APPLIED TO AN OPTIMIZED ALGATRON (QUARTZ-IODINE LIGHT SOURCE)

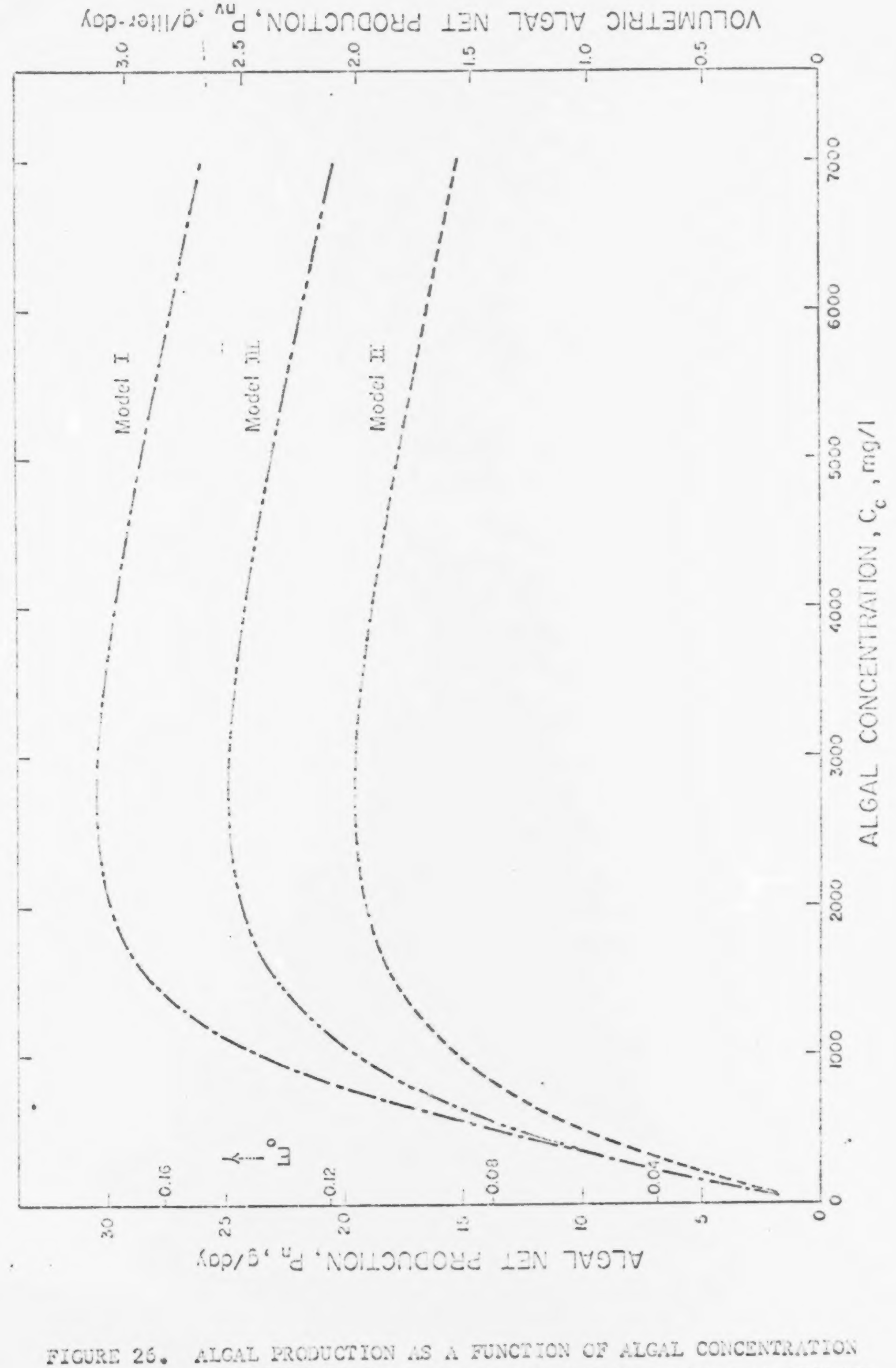


FIGURE 26. ALGAL PRODUCTION AS A FUNCTION OF ALGAL CONCENTRATION ACCORDING TO THE VARIOUS THEORETICAL MODELS AS APPLIED TO AN OPTIMIZED ALGATRON (QUARTZ-IODINE LIGHT SOURCE)

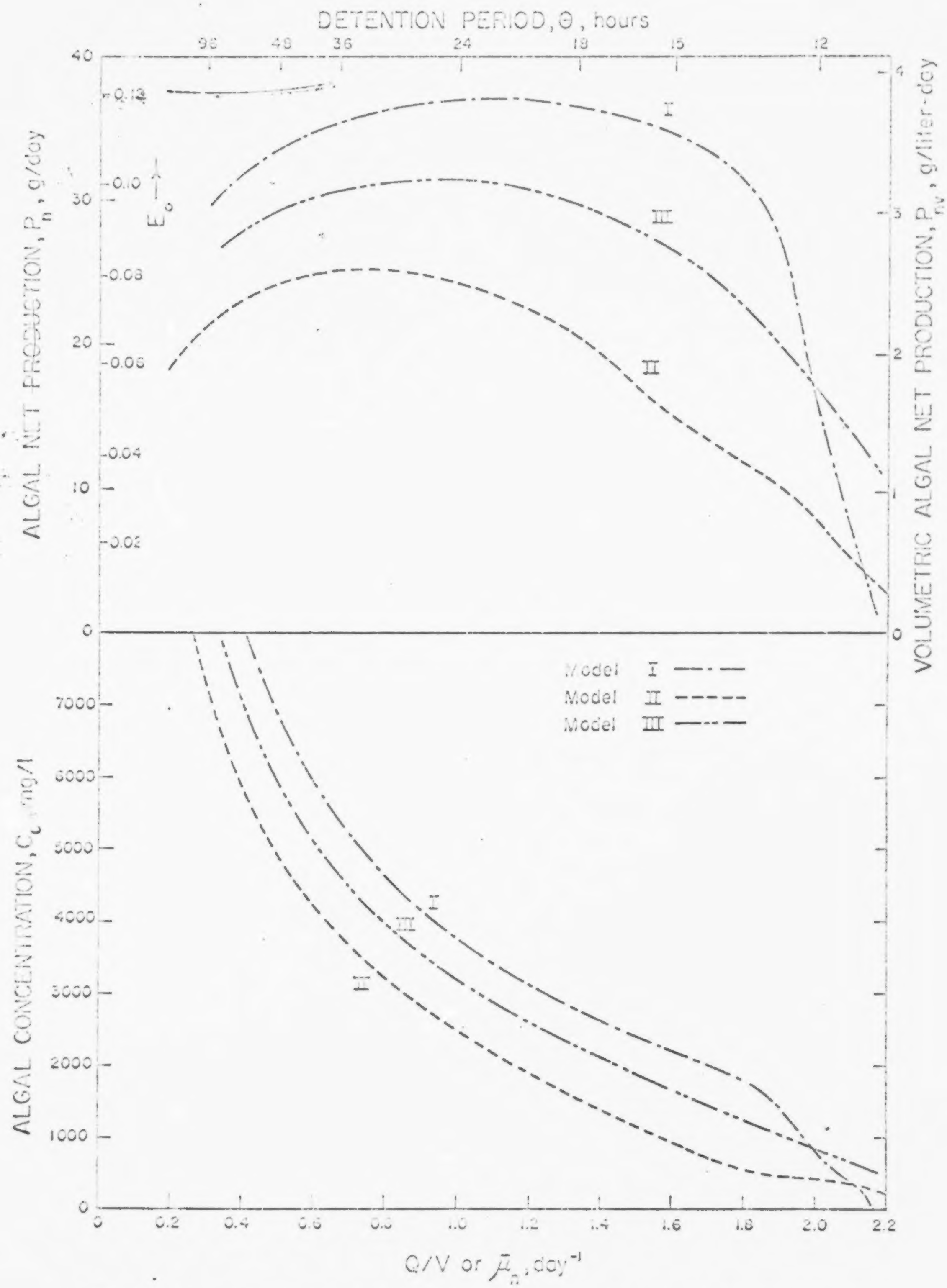


FIGURE 27. THEORETICAL KINETIC MODELS AS APPLIED TO AN OPTIMIZED ALGATRON (LUCALOX LIGHT SOURCE)

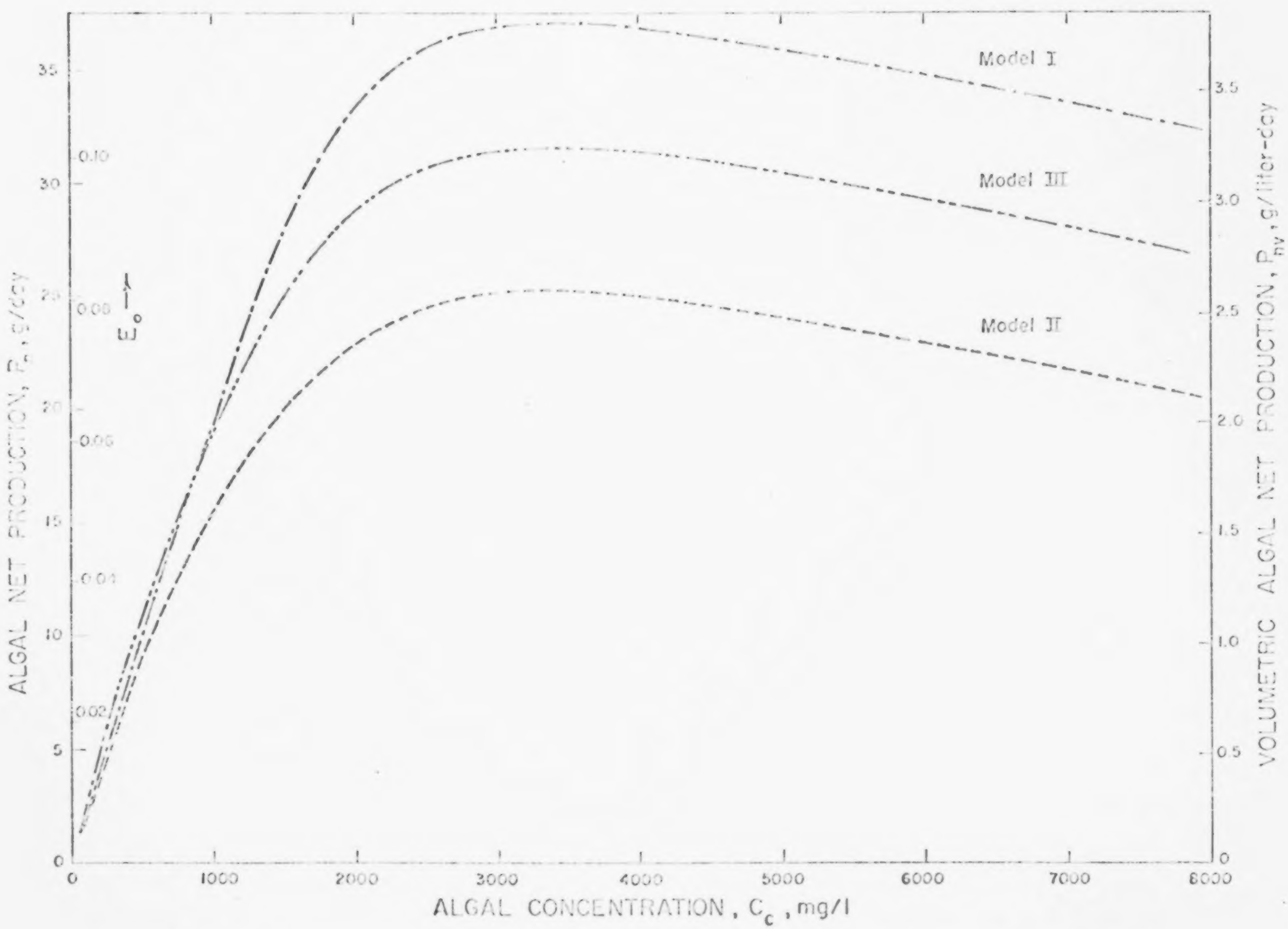


FIGURE 28. ALGAL PRODUCTION AS A FUNCTION OF ALGAL CONCENTRATION ACCORDING TO THE VARIOUS THEORETICAL MODELS AS APPLIED TO AN OPTIMIZED ALGATRON (LUCALOX LIGHT SOURCE)

PROPOSED MODIFICATIONS OF ALGATRON DESIGN

The current model of the Algatron has certain deficiencies in design which make it difficult to conduct the experiments required for evaluating completely the potential of the Algatron system for studying the kinetic characteristics of algal systems. As a part of an attempt to remedy this situation, a design was made for a new model in which would be incorporated features indicated as being desirable by the experience gained in work with the present model. The basic design of the proposed new model (culture volume, 2.5 liters) is shown in Figure 29. All necessary ancillary equipment not shown in the design would be incorporated in the model when it is constructed. Some of the basic features called for in the new design are discussed in this section of the report.

Variable Speed Drive

The current model has a fixed rotational speed of 210 rpm, which imparts to the drum a radial acceleration of 15.1 g. The new design calls for a variable speed motor to rotate the drum. Equipping an Algatron with a variable speed drive would make it possible to test the Algatron system under a variety of gravitational fields and centrifugal forces. An example of an advantage to be gained from this capability would be the ability to increase acceleration, and thereby flatten the slope of the culture film. Flattening the slope of the film would lead to a greater uniformity of light penetration. A second advantage would be the facilitation of a more thorough study of the flashing light effect than was possible with the present unit. By being able to vary the rotational speed of the culture, a wider range of time scales for flash and dark times could be applied than could be done with a fixed speed motor. A third advantage would be in the fact that power required to accelerate and drive the drum could be determined.

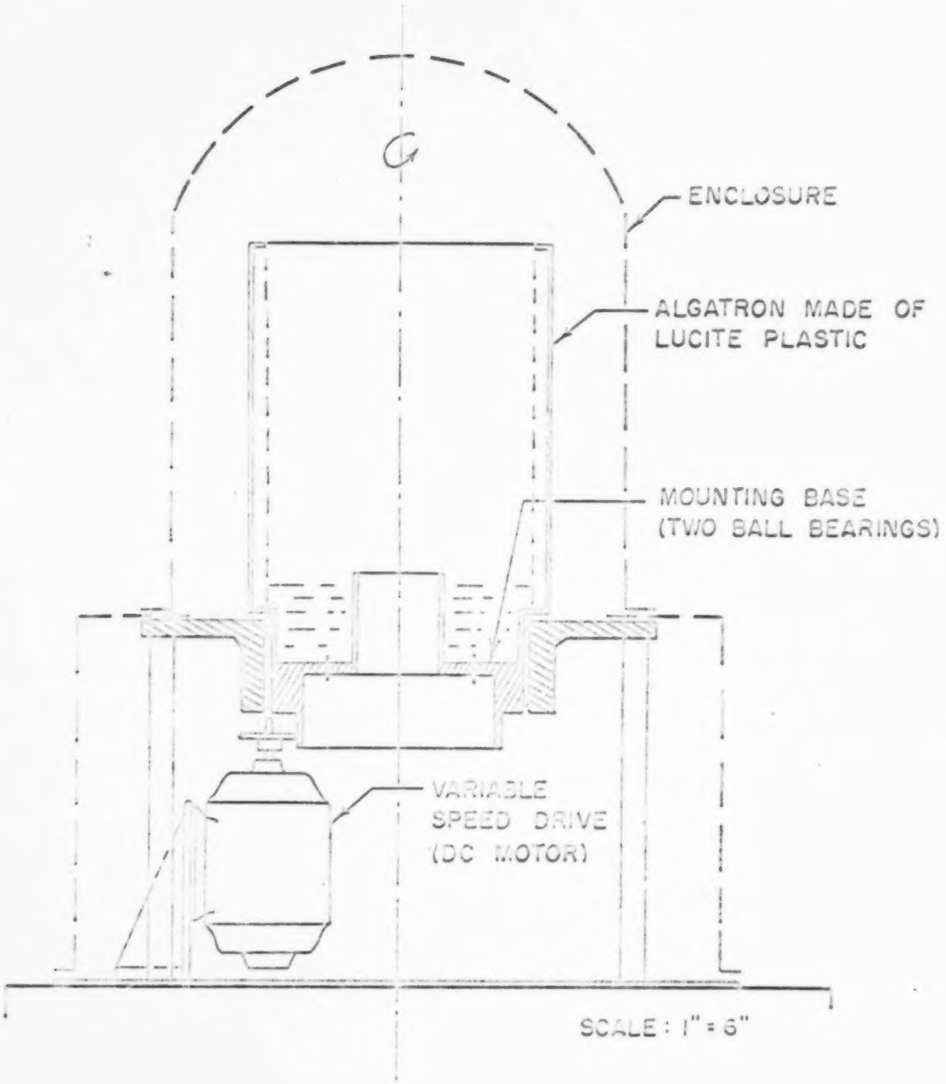

℄
ENCLOSURE
ALGATRON MADE OF
LUCITE PLASTIC
MOUNTING BASE
(TWO BALL BEARINGS)
VARIABLE
SPEED DRIVE
(DC MOTOR)
SCALE: 1" = 6"

FIGURE 29. Proposed Design for a Modified Algatron

Increased Ratio of Height to Diameter

The ratio of height to diameter in the current model is 1:2. Such a ratio results in an excessive consumption of space. It also is too low for the use of a light diffusing system of a type with which light conversion efficiencies could be increased. Indications are that a ratio of 1.4:1 would result in the elimination of these two drawbacks, and hence, it is this ratio applied to the new design.

Complete Enclosure

The current model as it now stands is not completely enclosed, and therefore accurate material balances such as those for gases and water must be done by indirect methods and certain corrections must be made. A complete enclosure would separate the system from the ambient environment, and thus make possible the precise maintenance of predetermined environmental conditions in the system, particularly adequate CO_2 concentrations to prevent carbon from becoming a limiting nutrient, and the accurate and complete determination of direct material and energy balances.

Turbidostatic Control

A chemostatic control was applied in the current Algatron prototype. In such a system a steady-state population density can be maintained. However, whenever a new dilution rate is applied, a substantial transitional period follows until a new steady-state level consonant with the new dilution rate is attained. The length of this transitional period will vary according to the genetic characteristics of the algae and the environmental conditions present. With a chemostatic control it also is very difficult to keep the algal concentration in the reactor at a constant level when varying experimental conditions such as applied incident irradiance or nutrient loadings.

The maintenance of a specific culture concentration is very important at times, particularly when the optical path of the light, amount of light absorption and transmission, and light energy balance are being studied.

An automatic density control (turbidostat) rather than a chemostatic control is included in the new Algatron design. Such a control would shorten the transitional time required for the formation of new steady-state following an adjustment in environmental conditions and would increase the overall experimental accuracy.

Reduced Reactor Volume

For future studies, a number of Algatron units of a reduced volume are recommended rather than a single relatively large unit. The current model with its capacity of 9.7 liters required the storage and delivery of a large volume of medium particularly when the higher dilution rates were applied. Judging from past experience, the individual unit need have a culture volume capacity of only 2.5 liters as far as laboratory-scale experiments are concerned.

Automatic pH Control

Automatic pH control is an obvious requirement for improving the capabilities of the Algatron. By having the capability of operating the system at any desired pH level, meaningful experiments can be made to determine the effects of pH on algal growth. Likewise, important studies on the relative availability of the various forms of carbon, i.e., carbon dioxide, bicarbonate, carbonate, which are functions of pH, can be performed. Concern over the buffer capacity of the media would be obviated.

Reflux Condensation System

The application of a reflux condensation system is required in order to prevent the high rates of evaporation normally attained and, thus, maintain a constant culture volume and hence constant experimental conditions.

Special Decanting Weir and Mixing Weir

By applying a special decanting weir, the need for recycling and the problems of effluent decanter clogging are obviated. Improvements in the mixing weir system are also indicated. CO_2 uptake and oxygen release are clearly influenced by boundary layer velocities at the air-water interface. The reciprocating plow is a vast improvement over single mixing probes, but the plow can be improved through special design of the blade system and perhaps through increasing the frequency of reciprocation and the degree of overturn of the fluid accomplished by the blades of the mixing device.

V. DESIGN OF OUTDOOR HIGH-RATE ALGAL REACTOR AND PRELIMINARY TESTS RESULTS

INTRODUCTION

Although studies on indoor laboratory models are well suited for determining the kinetic parameters governing algal growth, the limitations in applying the results of such studies indiscriminately in the design of full-scale outdoor models is obvious. The most important limitation is that, while indoor models can approximate a continuously stirred tank reactor (CSTR) very well, it is an almost impossible task to design an outdoor system on this basis without expending sufficient energy to make the cost of such a system economically unjustified.

Moreover, while the indoor studies reported during the course of this project were performed with essentially an algal phase and with either inorganic media or filtered sewage, real outdoor systems should be designed to operate on raw or primary treated sewage and would contain not only a significant bacterial phase, but a variety of zooplankton forms as well. Thus, outdoor systems must cope with the considerable suspended solids load and non-algal life present in these waste flows. To operate such a system as a CSTR would cause much light attenuation and absorption by suspended particles, thus reducing considerably the light conversion efficiency of the system and actually cause light limitations to severely hinder algal growth. Early work on high-rate ponds (95) indicated that mixing should be kept to a minimum, on the order of three hours per day. Results from the Interagency Study in Firebaugh, California (38), where high-rate algal systems are being evaluated for removing nitrate-nitrogen from the tile drainage of the San Joaquin Valley, have repeatedly shown that four hours or less of pond mixing per day gives optimum nitrogen removal efficiencies. The major reason outdoor ponds require only several hours of mixing per day is that when the pond is relatively quiescent suspended material settles to the bottom and algal growth roughly follows the relationships developed in Reference (45) for algae concentration vs. culture depth as a function of incident light intensity. However, in order to use these equations properly, integration of sunlight intensity at the proper wavelengths over a 24-hour period must be made. Also, conversion factors must be developed and added to the equations to account for non-algal turbidity and intermittant pond mixing as these factors affect sunlight availability to the algal cells.

Design of Outdoor Mini Ponds

In order to test and refine the kinetic models developed in the laboratory on real outdoor systems, four small test ponds, referred to as "mini" ponds, equal in all respects were constructed at the Sanitary Engineering Research Laboratory. These ponds were circular in design (7.5 ft. diameter), with a sidewall of 12 inches and an operating depth of 10-11 inches. The volume of the ponds was 275 gallons at 10 inches depth. They were constructed out of plywood with a fiberglass inner protective coating. In Figures 30 and 31 are shown schematic and plan views of the entire arrangement and in Figure 32 is shown a photograph of the four ponds.

Sewage Feeding System. The Sanitary Engineering Research Laboratory maintains a standard rate activated sludge plant complete with primary and secondary settling basins. Because of limitations on available land area, it was necessary to build relatively small growth units. The small size of

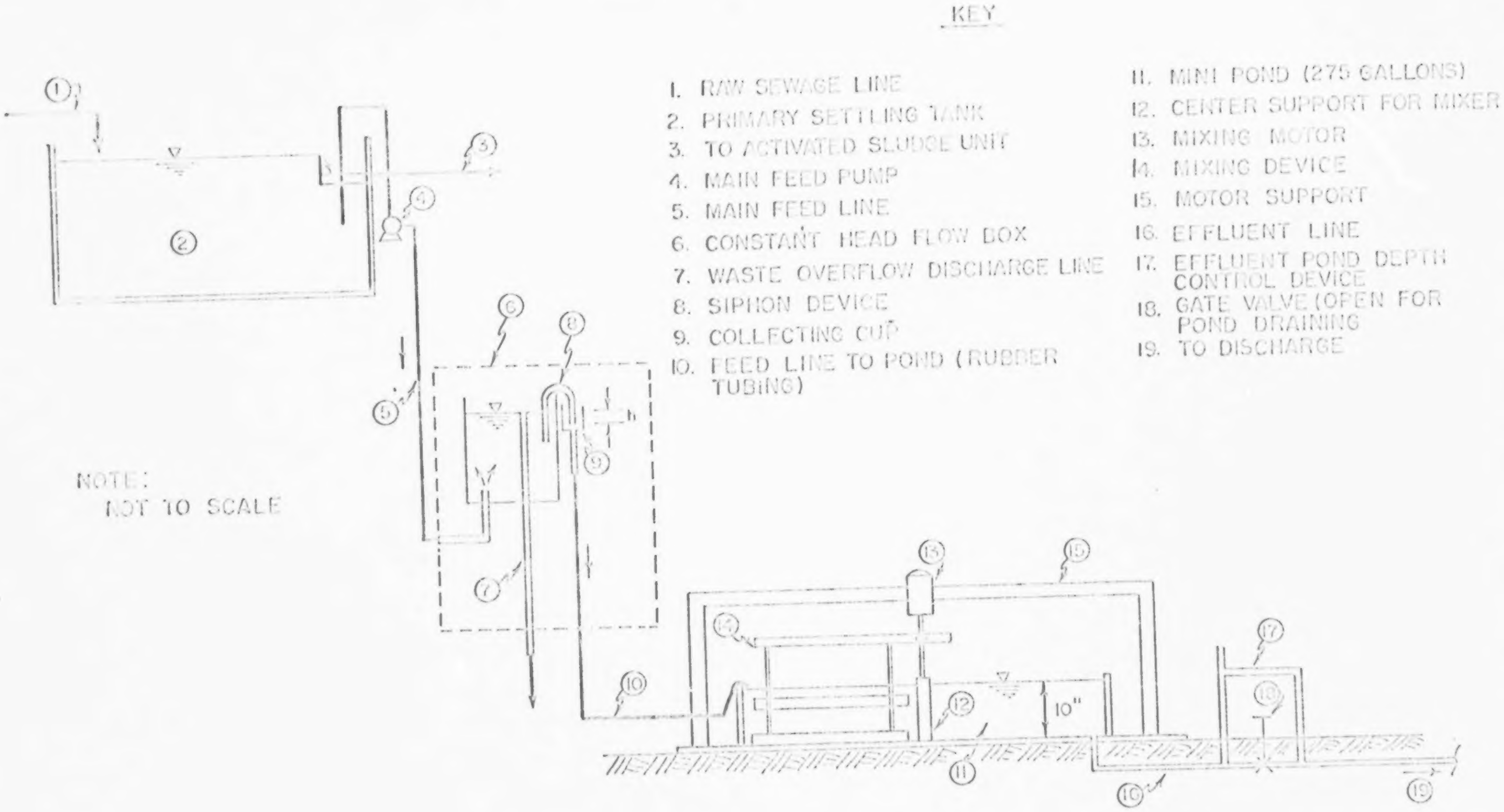

KEY
1. RAW SEWAGE LINE
2. PRIMARY SETTLING TANK
3. TO ACTIVATED SLUDGE UNIT
4. MAIN FEED PUMP
5. MAIN FEED LINE
6. CONSTANT HEAD FLOW BOX
7. WASTE OVERFLOW DISCHARGE LINE
8. SIPHON DEVICE
9. COLLECTING CUP
10. FEED LINE TO POND (RUBBER TUBING)
11. MINI POND (275 GALLONS)
12. CENTER SUPPORT FOR MIXER
13. MIXING MOTOR
14. MIXING DEVICE
15. MOTOR SUPPORT
16. EFFLUENT LINE
17. EFFLUENT POND DEPTH CONTROL DEVICE
18. GATE VALVE (OPEN FOR POND DRAINING
19. TO DISCHARGE
NOTE:
NOT TO SCALE
10"
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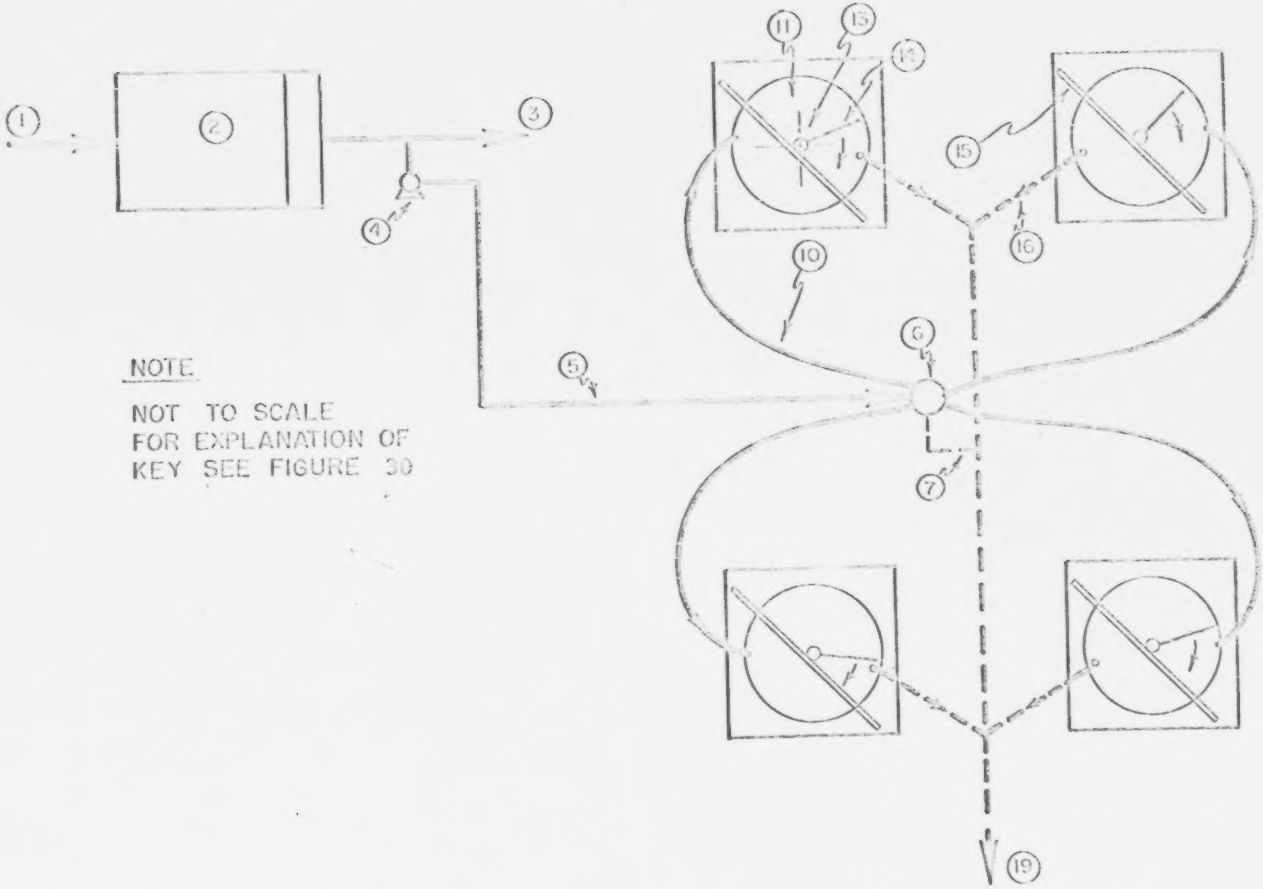
FIGURE 30. SCHEMATIC DIAGRAM OF MINI-POND ARRANGEMENT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NOTE

NOT TO SCALE
FOR EXPLANATION OF
KEY SEE FIGURE 30

FIGURE 31. PLAN VIEW OF MINI POND ARRANGEMENT

NOT REPRODUCIBLE

Figure 32 - Photograph of Mini Pond Arrangement

the ponds (275 gallons each at ten inches depth) resulted in very low flow rates at the residence times required in order to be consistent with high-rate pond practice. These residence times should vary between two and ten days depending on climatic conditions. A curve of required flow rates for this range of residence times at both 10 and 11 inches depth, as shown in Figure 33, demonstrates the sensitivity in residence time to small changes in flow rate at low residence times. Therefore, it was necessary to have an extremely accurate flow regulating system. In order to minimize the effect of clogging on the small diameter lines required to deliver such low flows, it was decided to use primary effluent as the source of sewage for feeding the pond.

After repeated failures with pumping devices for delivering the required range of flows to the ponds, a relatively simple arrangement was envisaged and successfully put into operation.

As shown in Figures 30 and 31, primary effluent is pumped in a 3/8" diameter galvanized pipe from the primary settling basin of the treatment plant 150 feet to a central head box located in the center of the mini pond area. The flow into the head box is always greater than the total maximum flow to be used in the four ponds. A constant head is maintained in the box by a vertical PVC pipe acting as an overflow weir. A drawing of this head box arrangement is shown in Figure 30. A U-tube is placed in position over the side of the head box with a distance "h" between the liquid level in the box and the discharge end of the inverted U-tube. A siphon is created in the inverted U-tube and liquid flows out at a rate which is dependent on the distance "h" and the diameter of the glass tube. By trial and error, a whole series of U-tubes capable of maintaining different desired flow rates was constructed. The liquid flowing out of these

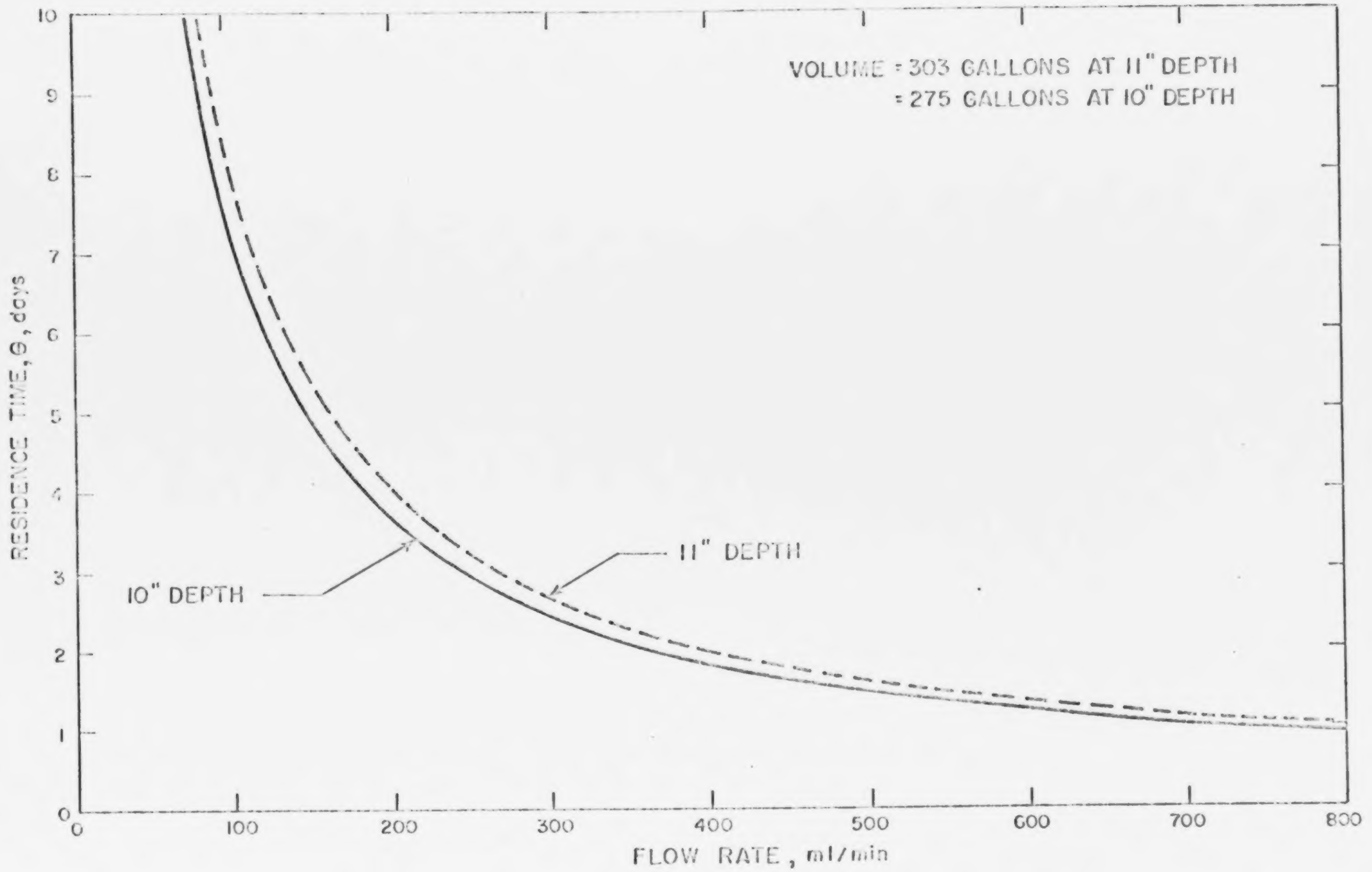

FIGURE 33. FLOW RATE THROUGH MINI PONDS AS A FUNCTION OF RESIDENCE TIME

U-tubes was collected in a plastic container to which quarter-inch rubber tubing was connected to an opening in the bottom of the container. The liquid flowed through this tubing by gravity to the pond where it discharged at the liquid surface. All excess liquid pumped to the head box was discharged over the overflow device. This system proved to be highly successful and only required a daily cleaning which took several minutes.

Mixing. Mixing was accomplished by a motor-driven rotating arm mounted on a center support to which was attached, by two vertical steel rods, two one and one-half inch wide blades, one placed near the pond bottom and the other near the surface. These blades were approximately three feet long and rotated in a radial fashion from the center of this pond. Thus, it was possible in one rotation to mix the entire contents of the pond. Attached to the bottom blade was a three-inch rubber strip which acted as a scraper to remove all settled material from the pond bottom. The motor, a Dayton 1/15 HP, right-angle gear motor, 2.8 RPM, was mounted from a horizontal beam (two by four) which traversed the pond and was itself supported at either end outside the pond by vertical columns (also two by fours). The vertical shaft of the motor was coupled to a steel rod which fitted through a quide hole in the center support. This support was made of a 1½" heavy duty PVC pipe, was connected to the steel vertical rod by drilling a hole through the pipe and slipping the rod through it. The arm was 5 ft. long with the vertical rod placed through the arm 1½ ft. from one end and the other 3½ ft. acting to support the mixing blades. The extra 1½ ft. of pipe length acted to balance the arm and relieve stress against the vertical rod. See the photograph in Figure 31 for a view of this arrangement.

The motor was attached to a timer so that any desired mixing schedule could be achieved. For the preliminary studies reported in this chapter, mixing was performed for one hour per day from 0800 to 0830 and from 1430 to 1500.

Effluent and Depth Control. Discharge from the pond was accomplished through a 3/4" hole in the pond bottom to which was connected a PVC pipe as shown in Figure 30. This pipe travelled underneath the pond floor and emerged outside the pond wall. It then formed a vertical inverted U with the height of the U 10 inches above the pond floor. This height controlled the depth of liquid in the pond. A bypass with a valve was connected at the base of this U so as to allow for gravity draining of the pond when required. The pond depth could be maintained at either 10 or 11 inches, depending on the height of the U. For the studies reported, the depth was maintained at 10 inches with 2 inches of freeboard.

Preliminary Results

A preliminary study was performed on nitrogen removal efficiency in the outdoor ponds (nitrogen removal is considered to mean nitrogen removal from solution by assimilation into algal biomass. It is assumed that any real high-rate pond will have a separation unit for removing the algae once grown).

The study was carried out for approximately one month from June 21, 1970 to July 24, 1970. The ponds were fed with primary treated effluent from the waste treatment plant. The initial algal innoculation was a concentrate (10% solids) of *Scenedesmus quadricauda* obtained from the Interagency Agricultural Waste Water Treatment Center in Firebaugh, California. A heavy dose of this concentrate was added to three of the four ponds on June 17. These ponds were allowed to operate on a batch basis for several days until a significant algal biomass was established and then operated on a continuous flow bases. The fourth pond was started up a few weeks later. Mixing was accomplished from 0800 to 0830 and from 1430 to 1500.

The ponds were operated at residence times of 2, 2.5, 3 and 5.5 days. The ponds were sampled and analyzed twice a week for the following chemical characteristics: total unfiltered nitrogen, total filtered nitrogen, filtered ammonia, nitrite and nitrate nitrogen and algal biomass as measured by volatile solids. No attempt was made to differentiate between algal and non-algal biomass and these determinations must, therefore, be considered to be only a crude estimate of the amount of algae present. All determinations were made according to SERL Analytical Methods (76).

After approximately one month of operation, the ponds appeared to reach steady-state. In Figure 34 is shown nitrogen removal efficiencies for the four systems at this time. Total nitrogen entering the ponds was approximately 25 mg/l as determined by daily composite total nitrogen determinations. Approximately two-thirds of this nitrogen was made up of ammonia nitrogen and the remaining third was organic nitrogen. Only trace amounts of nitrite and nitrate nitrogen were present in the pond influents. As can be seen from Figure 34, residence time did not appear to have any effect on nitrogen removal efficiencies as all ponds were removing from solution over 90 percent of the influent nitrogen.

Several possible explanations to describe this occurrence are: (1) the pond systems were far from being CSTR type units. Thus, the true residence times were far from their designated values. (No attempt was made at this time to study the hydraulic characteristics of the ponds, particularly the degree of short-circuiting). (2) A great deal of the ammonia nitrogen was being evolved to the atmosphere as NH_3 due to high daytime pH values (10-11) experienced; (3) nitrogen might have been tied

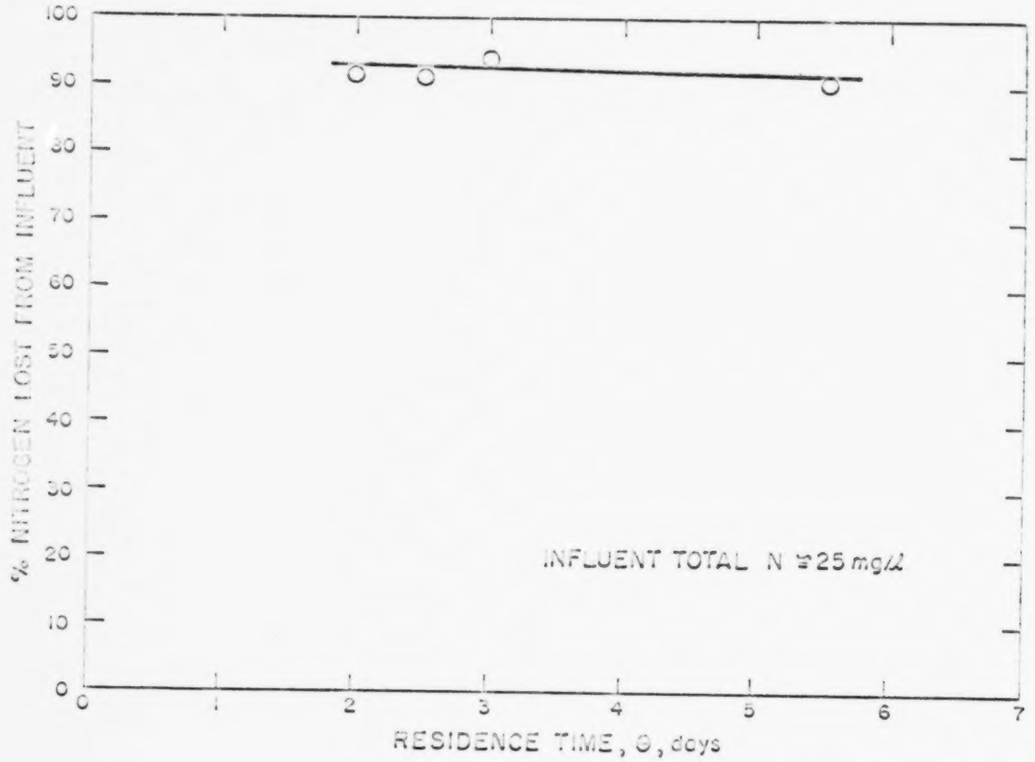

FIGURE 34. NITROGEN REMOVAL FROM SOLUTION AS A FUNCTION OF RESIDENCE TIME IN OUTDOOR MINI POND EXPERIMENT

up in the sediments formed at the pond bottom and an accurate nitrogen mass-balance determination would reveal less real nitrogen removal efficiencies than was determined by measuring soluble nitrogen in the influent and effluent and using these values to calculate a removal efficiency.

These results must, therefore, be interpreted very carefully and no conclusive statements can be drawn from them without further and more elaborate experimentation. These results do, however, suggest that algal systems are very efficient at removing nitrogen.

this specie and algae. At present (and for the past several years), the large high-rate oxidation pond at the Sanitary Engineering Research Laboratory (SERL) in Richmond, California, is infested with Potamocypris, almost to the exclusion of all algae. This pond served as a stock for all ostracods used in the experiments. It also served as an example of a viable habitat for the ostracods in comparisons with four smaller operational high-rate ponds also located at SERL.

Very little work has been done on the ecology of ostracods, probably due to the variability of characteristics among the many genuses. This investigation was a small contribution to an area in need of much more work.

To date only a few studies have been done on predators which occur in an oxidation pond. Oswald (95) studied the effect of rotifers on BOD removal and showed that removal was hampered by an infestation. He also found rotifers to be able to withstand the range of dissolved oxygen and pH normally found in an oxidation pond. McGarry in Thailand (96) found that a high BOD loading prevented infestations of predators. In South Africa, Tchortner (97) presented detailed ecological studies of two oxidation ponds concentrating on the algae and crustacean populations. An extensive analysis of protozoan and metazoan distribution and succession has been done by Alagarsamy et al. in India (98). They found Rhizopodes and Flagellates predominating at the start, being replaced by the free-swimming citiates. Finally, Porcella (99) has studied the growth factors of Daphnia, another common predator in oxidation ponds using Strontium-90.

EXPERIMENTAL PROCEDURES AND RESULTS

As a preliminary experiment, 100 Potamocypris were filtered from the inoperative ponds and put into each of 12 beakers containing 500 ml of liquid from the operational smaller ponds (see Chapter V for a description

VI. ALGAE-OSTRACOD RELATIONSHIPS IN HIGH-RATE OXIDATION PONDS*

INTRODUCTION

Studies on the biology of oxidation ponds are often concerned only with the symbiotic mechanism between algae and bacteria. Due to the abundance of unoccupied ecological niches in an oxidation pond, however, higher forms of life often appear and predator-prey relationships become important to the ecology of the pond. Predators directly effect the growth and endurance of algae and indirectly the general performance of the pond. By focusing attention on the environmental parameters affecting these predators, a great understanding of their total ecology can be gained and steps can be taken toward their removal. Since these steps will be biological in nature, the temptation to introduce pesticides will be lessened. Moreover, by understanding the mechanisms at work, an environment inviable to the predators can be maintained and an infestation of predators can be prevented from the start.

It is the aim of this report to present the results of an investigation of environmental parameters suspected of influencing the growth and viability of the predator, Potamocypris, an ostracod of Western United States, and based on these results, to formulate a tentative mathematical model describing the dynamics of the predator-prey relationship between of these ponds), commonly referred to as "mini ponds", and 500 ml of varying effluents (i.e., raw, primary, activated sludge and final) from the SERL waste water treatment plant. The mini ponds had a well-developed culture of Scenedesmus quadracauda at the time. After one day approximately 90% of all the Potamocypris were dead. Since Potamocypris had survived previously in the various effluents, death was attributed to some factor related to the heavy dose of Scenedesmus. Accordingly, a plan of study was formulated to compare environmental conditions in the inoperative pond and the mini pond and to test for a range of tolerance in Potamocypris for two of these factors--dissolved oxygen and pH, since both of these factors are greatly influenced by algal growth.

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*This chapter was written by Joan E. Harrison, Undergraduate Ecology student at Tufts University, Medford, Mass., who spent the summer of 1970 conducting research at the Sanitary Engineering Research Laboratory of the University of California, Berkeley, on a fellowship from the National Science Foundation Undergraduate Research Program.

Dissolved Oxygen

To test for viability at different concentrations of dissolved oxygen, tap water was used which ranged from 1.4 mg/1 of oxygen to 38.9 mg/1 of oxygen. Removal of the oxygen in 2 liters of tap water was done by bubbling pure nitrogen through the liquid at three lbs. pressure for two hours, while supersaturation was obtained by bubbling pure oxygen through four liters of tap water for two hours at three lbs. pressure. A range was obtained by dilution with tap water. Potamocypris was filtered from the inoperative ponds into tap water and a pipette-full (50-100 ostracods) was put into each of two 250 erlenmyer flasks and immediately filled to the brim with the oxygenated or deoxygenated water and stoppered. At the same time, a DO bottle was filled with the same level of DO water and the exact amount of dissolved oxygen was determined by the Winkler method (76). After 24 hours the Potamocypris were counted under a dissecting microscope. In Figure 35 is presented an average percentage of living Potamocypris for each level of dissolved oxygen. The pH for this study ranged betwen 8 and 9.

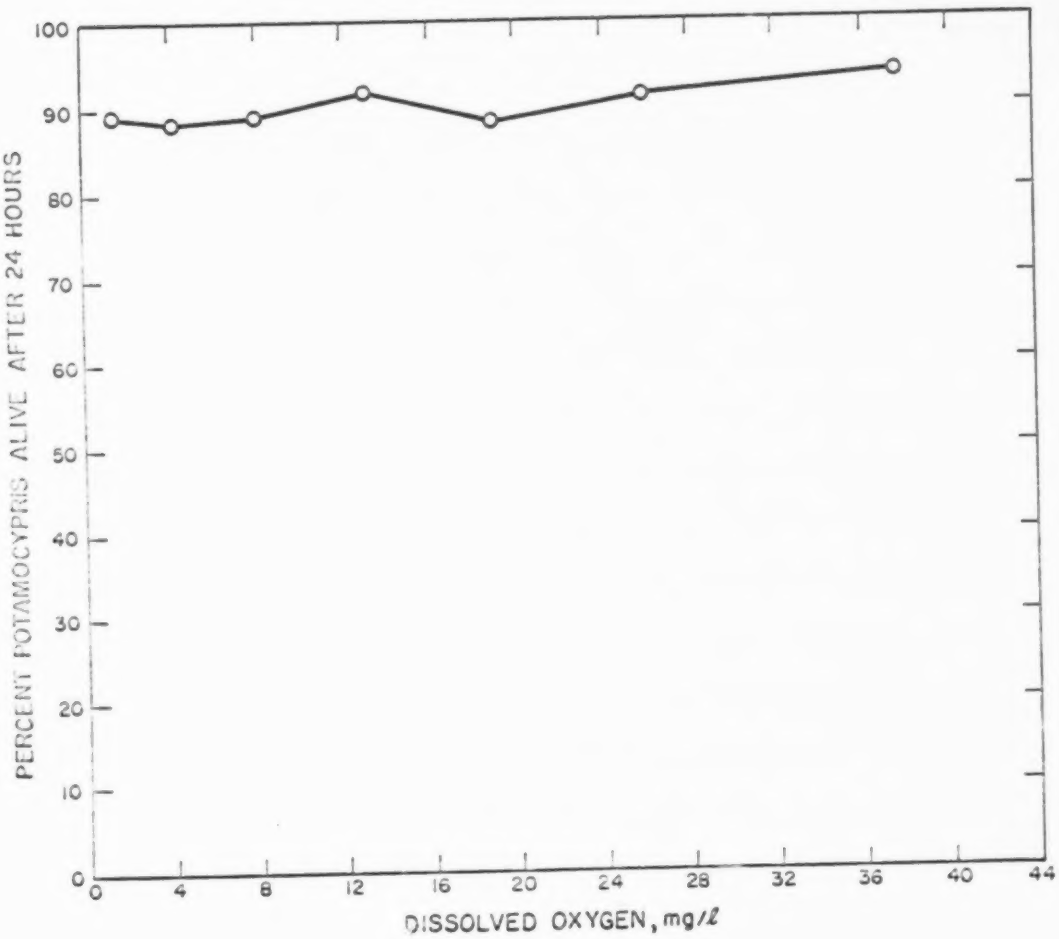

Figure 35. Viability of Potamocypris with Respect to Varying Dissolved Oxygen Concentrations.

As can be seen in Figure 35, the percentage of survival was above 85% at all seven levels of dissolved oxygen. The average deviance from a mean of 90.5% was only 1.8%. Apparently, extreme differences in levels of dissolved oxygen make little difference to the survival of *Potamocypris*.

In order to determine the *in vivo* range of dissolved oxygen encountered in both a favorable and an unfavorable habitat for *Potamocypris*, a diurnal study was performed to characterize dissolved oxygen levels in both the inoperative and the operative mini ponds. Samples were siphoned from near the bottom (the area inhabited by ostracods) every two hours for 22 hours from the inoperative pond and all four mini ponds and the DO was determined. The levels of oxygen shown in Figure 36 do not exceed the range of those found to be viable for *Potamocypris* in the previous experiment. It can be surmised that DO is not a deciding factor in determining the suitability of a habitat for this species of ostracod and therefore will have little application in controlling *Potamocypris* in a high-rate oxidation pond.

pH

In testing for pH affects on *Potamocypris*, jars with 150 ml of tap water were titrated to unit levels of pH from 4 to 12 inclusive. Approximately 50 *Potamocypris* were put in each jar and covered. After five hours, the pH's were checked and restabilized. After 24 hours, the pH's were again checked and the ostracods were filtered and counted to determine the average percentage alive for each level of pH. These percentages, shown in Figure 37, seem to delineate a range of pH tolerance of six through ten. Of particular interest is the sharp decline from 10 to 11. The pH of the mini pond water in which the *Potamocypris* could not habitate in the preliminary experiment was 10.85. Thus, there was a strong indication that

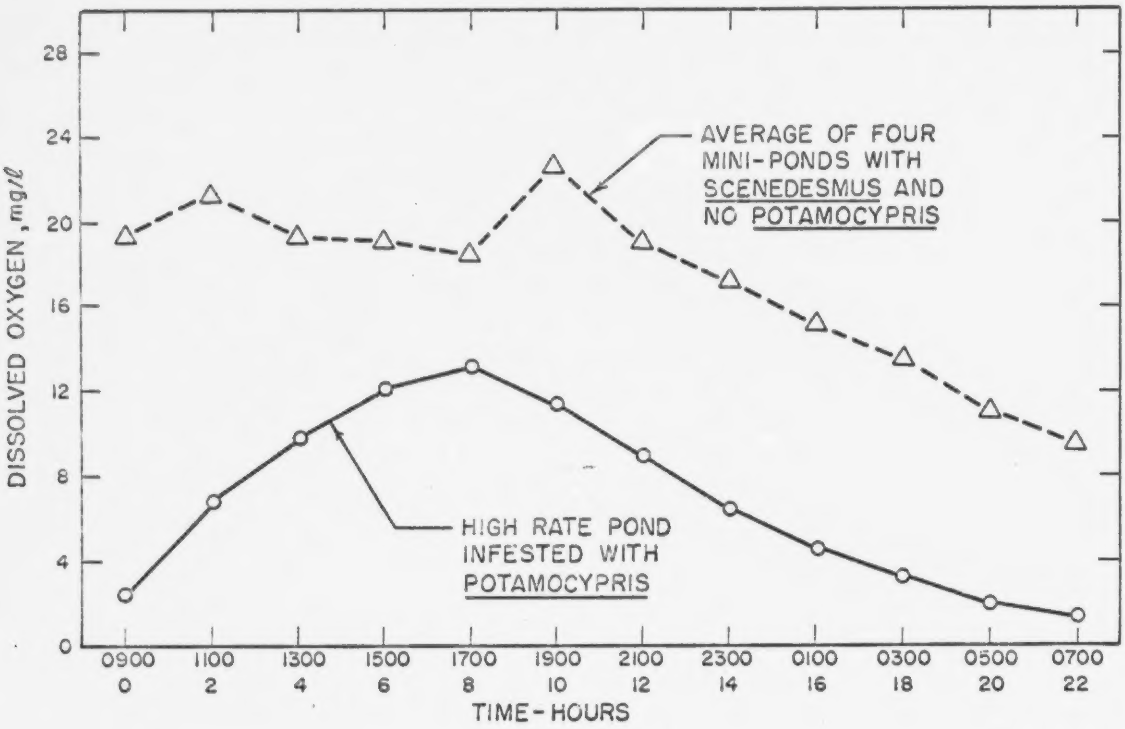

Figure 36. Diurnal Changes in Dissolved Oxygen Concentration in High-Rate Ponds.

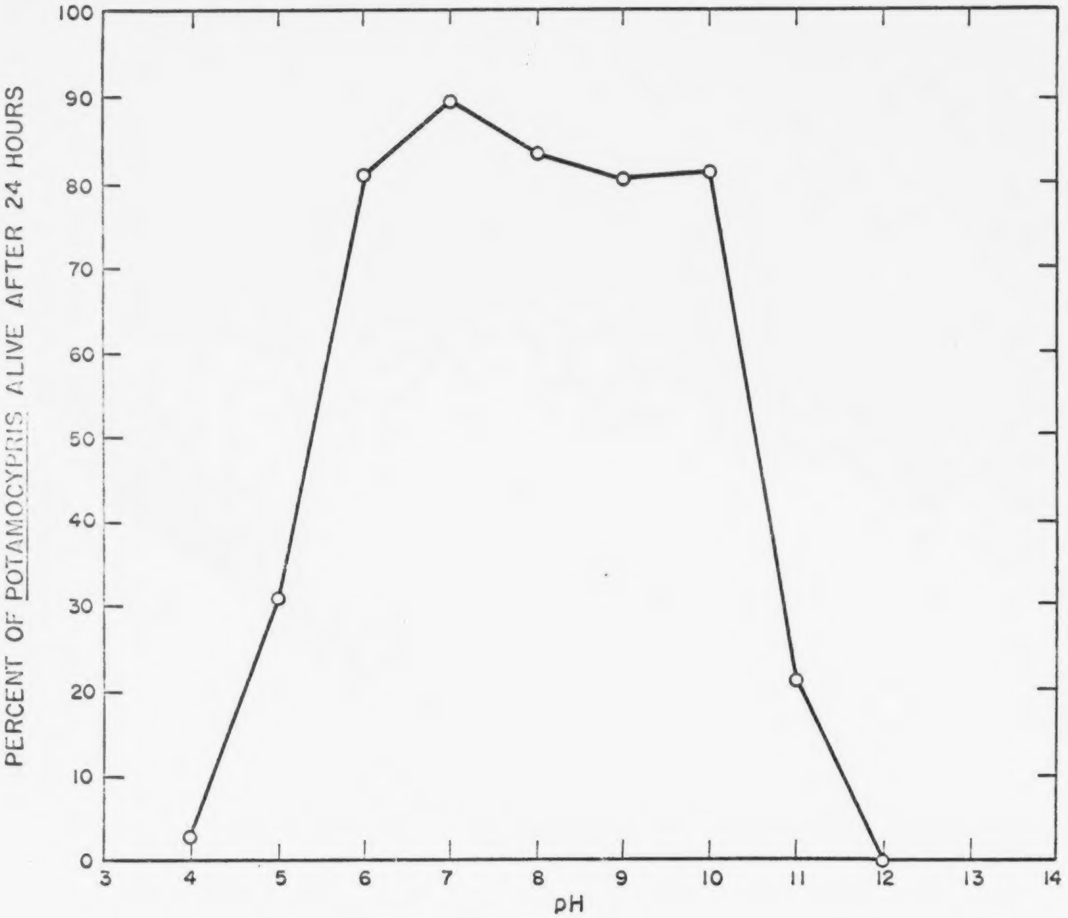

Figure 37. Viability of Potamocypris with Respect to Varying pH Levels.

this species is unable to survive at pH values greater than 10.

A diurnal study was done on the inoperative pond and the four mini ponds to determine the range of pH's encountered in both favorable and unfavorable habitats. The study was done in the same manner as the dissolved oxygen diurnal study, with samples siphoned every two hours for 24 hours. The pH's were determined on a Leeds and Northrup general purpose laboratory pH meter. The results can be seen in Figures 38 and 39.

There is a wide separation of the diurnal curves of pH encountered in an inoperative pond and a well-working pond. With a low pH of 7.7 and a high of 8.8, the favorable habitat fluctuates around a mean of 8.2. The unfavorable habitats have a high pH of 10.9 and a low of 9.6 with the mean being at 10.3. The wide separation of pH ranges could be a deciding factor in the favorability of a habitat, especially since it was found before that there is a sharp drop in Potamocypris viability after a pH of 10. A more detailed look at the pH ranges of the individual mini ponds is shown in Figure 39. There it can be seen that two of the ponds have a pH range that stays above 10 for almost the entire day. All have a peak pH of about 10.9 at about 7:00 PM. Because Potamocypris may not be able to survive at these high pH's encountered, there is the possibility that pH could be an important factor in the control of Potamocypris.

In testing for a pH tolerance range, there was a chance that the rapid die-off was due to the sudden change in pH. An experiment was designed to test for physiological adaptation with slow rises in pH. The viability of Potamocpyris was measured at pH's of 8 to 12, but only after a number of days at a particular pH depending on the distance from the starting point of 8 (e.g. Potamocypris to be tested at a pH of 9 were held one day at 8 and one day at 9 and counted, while those to be counted at a

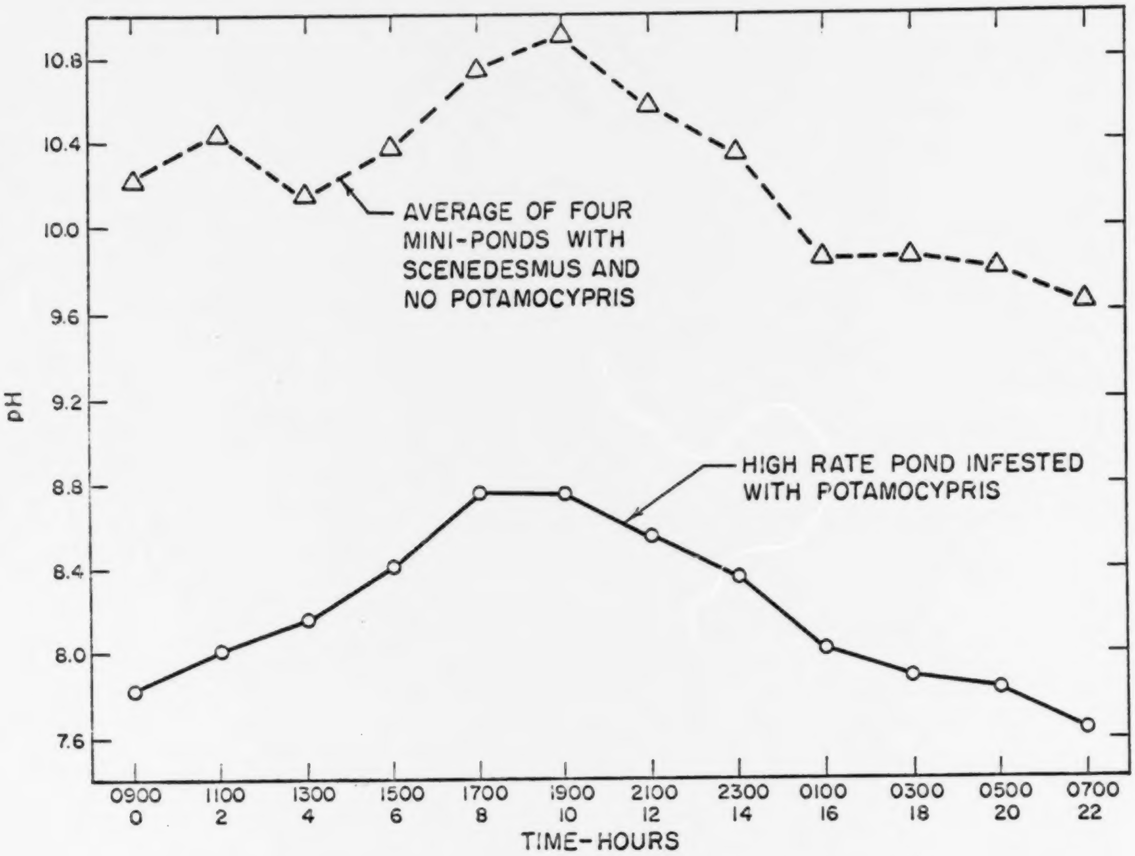

Figure 38. Diurnal Changes in pH in High-Rate Ponds.

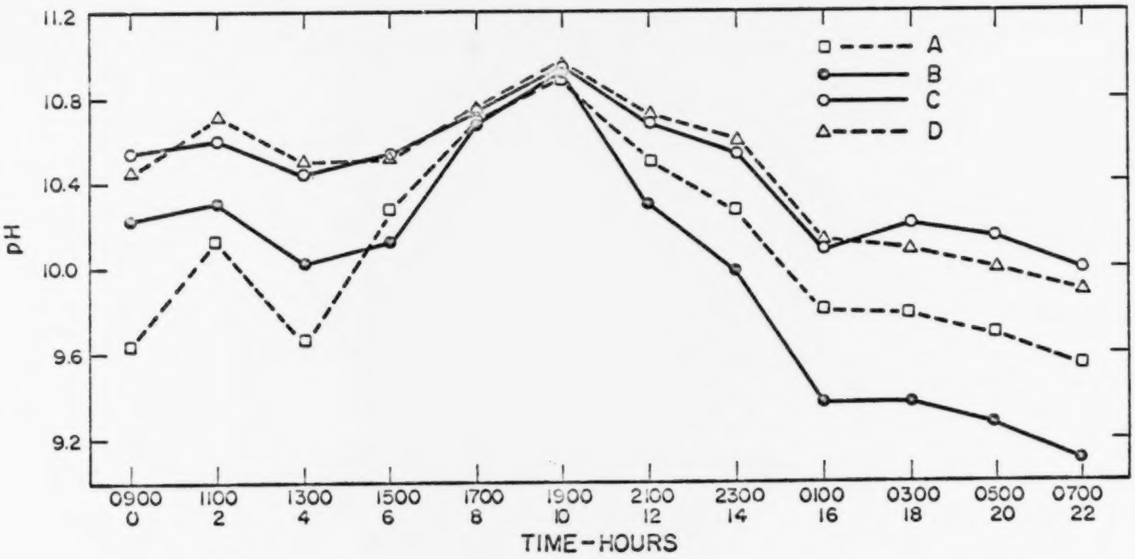

Figure 39. Diurnal Changes in pH for Separate Mini Ponds.

pH of 12 spent five days increasing to that point). For each level of pH, four jars with 150 ml of tap water were used, each being titrated to a pH of 8 with 0.1 N H_2SO_4 at the start of the experiment. 0.01 N NaOH was used to raise the pH to its designated value. After the four jars had stayed for 24 hours at the designated pH, two were counted and two were restabilized and left until the end of the five-day experiment. This pattern was followed on successive days with lower pH's being stabilized, two of the jars being counted, and jars to be counted at higher pH's titrated to the next unit of pH. At the end of a five-day period, the restabilized jars at the lower pH's were counted as well as those that had remained at a pH of 12 for 24 hours. Thus, those at a pH of 8 were maintained at that pH for a period of five days, those at a pH of 9 were maintained at 8 for one day and at 9 for four days. This procedure provided a method for eliminating the effect of time as a cause for death at the higher pH's.

In Figure 40 the percentage Potamocypris alive after 24 hours at each pH after a slow rise is compared with percentages previously found at each pH without a slow rise. As can be seen by the closeness of the curves in the two graphs, there seems to be very little physiological adaptation to changes in pH. The one significant possibility of physiological adaptation is at a pH of 11 where the percentage of survival after 24 hours was 35.9 as compared to the previously found percentage of 23.1 after 24 hours at pH 11. These percentages do not include the two out of every four that were left as controls until the end of the five days. Besides serving as controls, these other jars provided some indication of the effect of time for some of the pH levels. The percentage found after counting these jars at the end of five days is shown in Figure 41 along with the graph previously shown in Figure 40. Next to the points of each control is the

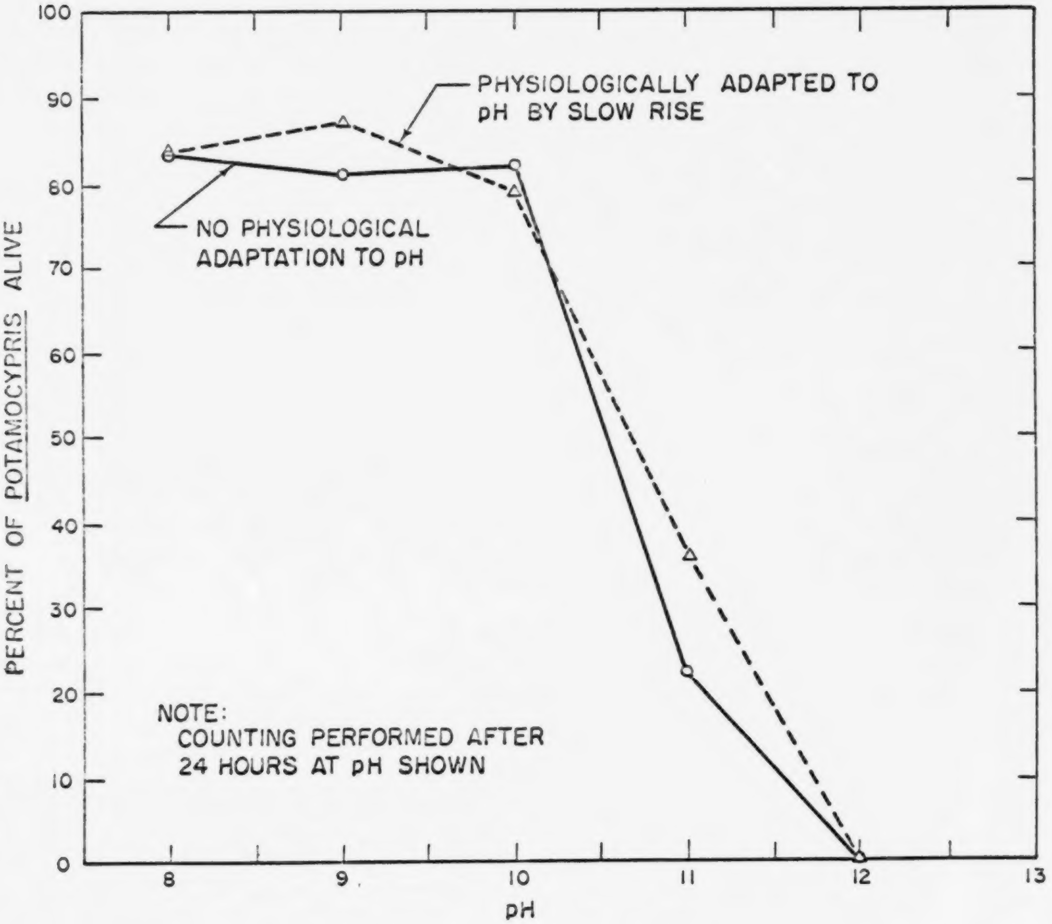

Figure 40. Viability of Potamocypris with Respect to Changes in pH with and without Physiological Adaptation.

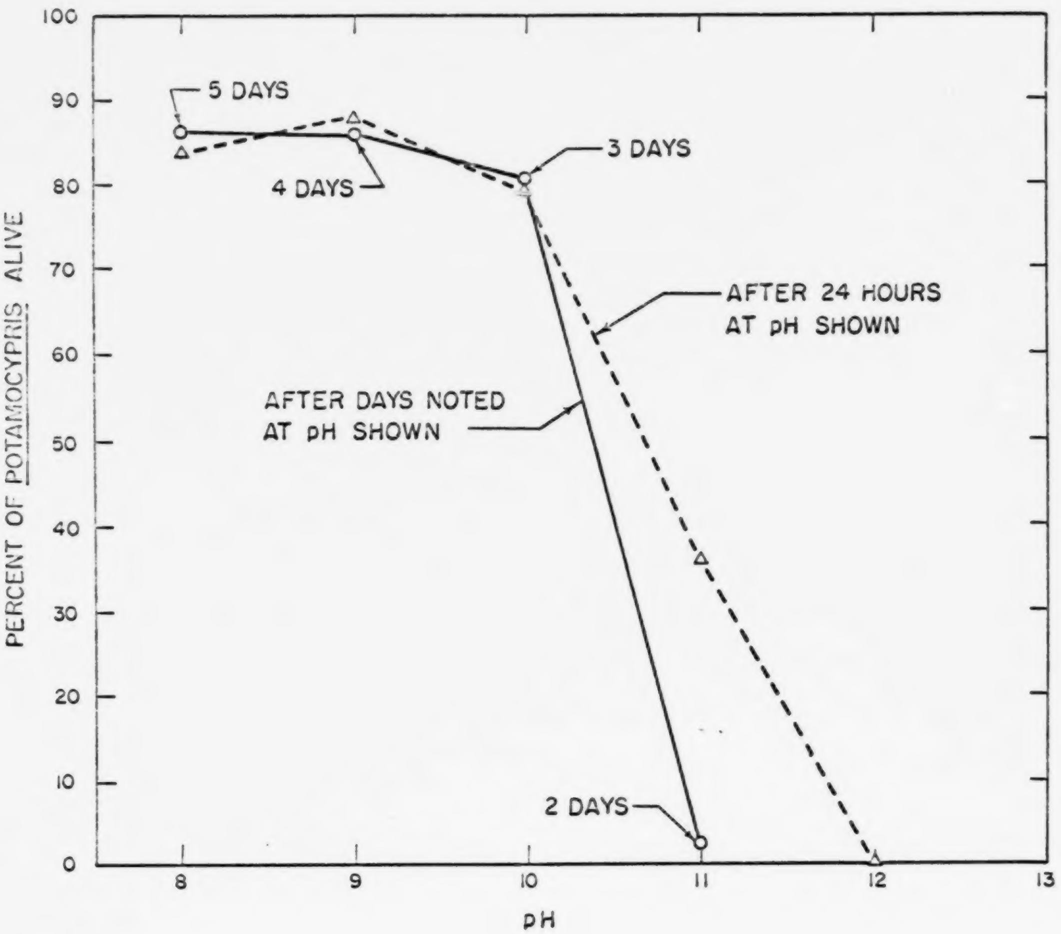

Figure 41. Effect of Time at a Particular pH and Physiological Adaptation on *Potamocypris* Viability.

number of days that the particular pH was maintained after having gone through the slow rise. Based on these results, it seems that the effect of more than one day at a pH does not appear to have an effect on viability at levels of 8-10 but does have a detrimental effect at a pH of 11 where the count was decreased from 35.9% after 24 hours to 3.2% after 48 hours. If this effect begins after a pH of ten, it could be of importance in maintaining a predator-free, well-working pond at steady state.

The possibility that the inability of the Potamocypris to survive in the water titrated to pH's above ten was due to the conductivity and not the pH was dismissed when the conductivity of the NaOH titrated water used for a pH of 11 was found to be 1.0 millimhos as compared to the water taken from the favorable habitat which measured a conductivity of 1.1 millimhos.

Diurnal studies of temperature and conductivity are shown in Figures 42 and 43.

THE MATHEMATICAL MODEL

Based on these results it seems that pH has an effect on the survival of Potamocypris. To use this information for the control of these predators, an understanding is needed of the entire predator-prey relationship and how it is involved with this effect of pH. Such an understanding would provide a means for predicting the effect changes in pH can have on the system. Accordingly a mathematical model of the effect of pH was formulated based on a simplified view of the population dynamics.

Several initial assumptions were made for this model: 1) the predator-prey interaction is confined to the Potamocypris eating the algae and the algae raising the pH by photosynthesis (and resulting shifts in the CO_2-bicarbonate equilibrium system to the alkaline range) to levels toxic to

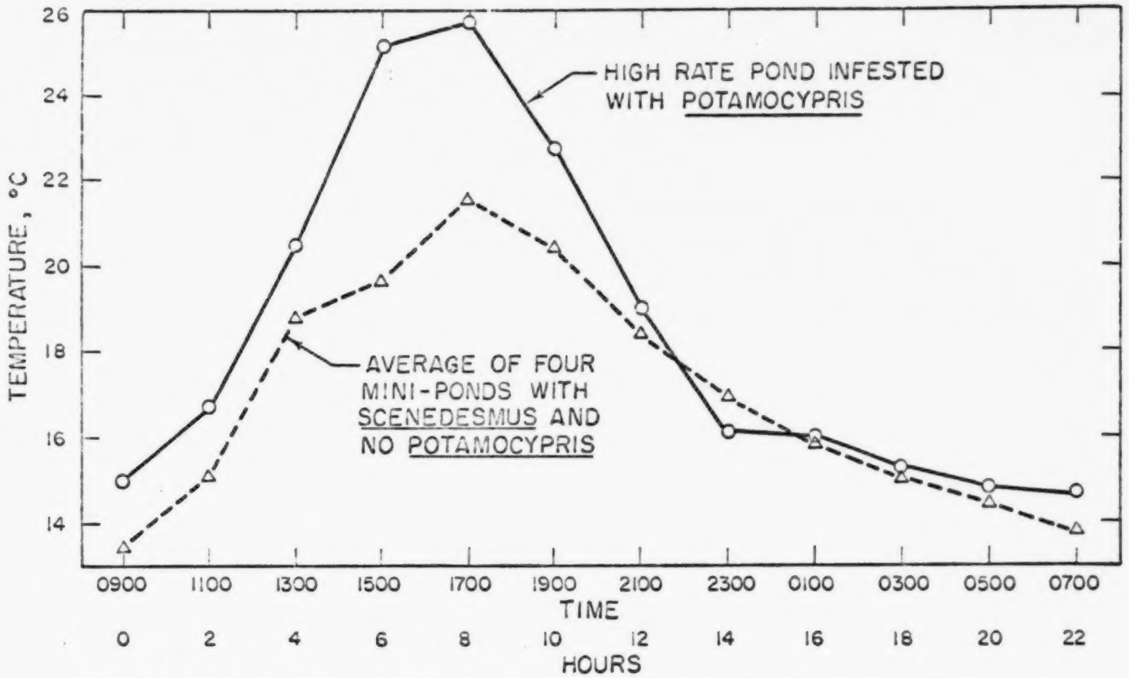

Figure 42. Diurnal Changes in Temperature for High-Rate Ponds.

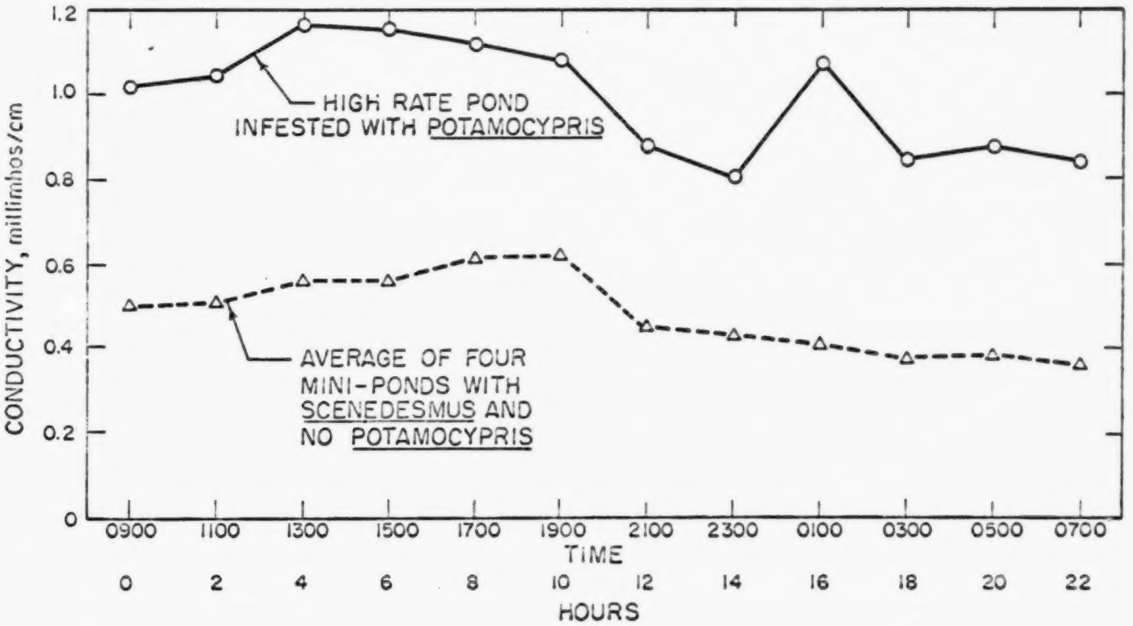

Figure 43. Diurnal Changes in Electrical Conductivity for High-Rate Ponds.

Potamocypris; 2) Other than their respective birth and death rates, all other influences on the population of algae and Potamocypris are excluded; and 3) The pH is a function of the concentration of algae. Based on these assumptions, it can be hypothesized that there is some critical ratio of algae to Potamocypris, R_C, such that if the ratio of initial concentrations R_I is greater than R_C, the population of algae will increase to a maximum to the relative exclusion of the Potamocypris. On the other hand, if the initial ratio of concentrations is less than the critical ratio, the population of Potamocypris will increase to a maximum to the relative exclusion of the algae. The critical and initial ratios can be described as:

$$R_C = \frac{[N_{1\ crit}]}{[N_{2\ crit}]} \qquad R_I = \frac{[N_{1\ init}]}{[N_{2\ init}]} \qquad (6\text{-}1)$$

where N_1 = concentration of algae-weight basis

N_2 = concentration of Potamocypris-weight basis.

If $R_I > R_C$, then we hypothesize that algae predominate, whereas, if $R_I < R_C$, then Potamocypris predominate.

Another way of describing this situation would be: Given an initial concentration of Potamocypris, if enough algae were introduced so that they raised the pH above 10, the Potamocypris would die off faster than they could eat the algae.

The equations describing the prey-predator relationship are based on the relationships set forth by Gause (100), which relate the change in population of prey per unit time, $\frac{d\ N_1}{d\ t}$, to the change in population of predator per unit time, $\frac{d\ N_2}{d\ t}$. Gause's equations give rise to oscillating systems where each population is density dependent on the other because of their interrelationship. With Potamocypris, however, we may suspect that no oscillations will occur because the ostracods of this and many other genuses have many alternative sources of food and these are not limited by a decline in algae. Pennak (101) suggests that ostracods filter feed any form of organic matter within a certain size range. In the experiments previously reported, Potamocypris were observed feeding off the internal parts of dead Potamocypris. Thus, it can be assumed that the Potamocypris has an unlimited food source. The algae, on the other hand, are directly affected by the density of the Potamocypris. The equation describing the algal density changes is assumed to be:

$$\frac{d\,N_1}{d\,t} = b_1\,C_1\,N_1 - d_1\,C_2\,N_2 \qquad (6\text{-}2)$$

That is, the change in population of algae per unit time is dependent on the birth rate, $b_1\,N_1$, with a limiting term, C_1 (which results from the assumption of a maximum concentration due to light or other limiting factors), and the death rate, $d_1\,N_2$ due to consumption by the population of Potamocypris. C_2 is a factor which relates the concentration of algae to the rate of consumption after Ryther (102).

Given (1) that the limitation of growth of algae at some maximum density is inversely related to some power of the ratio of (N_1) to a maximum, then

$$C_1 = 1 - \left(\frac{N_1}{N_{1M}}\right)^{e_1} \qquad (6\text{-}3)$$

where N_{1M} = maximum concentration of algae

and (2) that the factor relating algae concentration to consumption C_2 is inversely related to a ratio developed by Ryther, then

$$C_2 = 1 - \left(\frac{N_{1L}}{N_1}\right)^{e_2} \qquad (6\text{-}4)$$

where N_{1L} = lower limiting algal concentration.

The equation for changes in algal population as a function of time then becomes:

$$\frac{d\ N_1}{d\ t} = b_1 \left[1 - \left(\frac{N_1}{N_{1M}} \right)^{e_1} \right] N_1 - d_1 \left[\left(1 - \frac{N_{1L}}{N_1} \right)^{e_2} \right] N_2 \qquad (6\text{-}5)$$

The change per unit time in the Potamocypris population is dependent on the concentration and is affected by three factors--the birth rate, b_2N_2, with a maximum population limitation, C_3, the natural death rate, d_2N_2, and the death rate, $d_3C_4N_2$, related to pH effects which are some function of algal concentration.

Then, the equation describing changes in Potamocypris population as a function of time is:

$$\frac{d\ N_2}{d\ t} = N_2\ (b_2\ C_3 - d_2 - d_3\ C_4) \qquad (6\text{-}6)$$

Given that (1) the limitation of maximum density is inversely related to a power of the ratio of N_2 to a maximum, then

$$C_3 = 1 - \left(\frac{N_2}{N_{2M}} \right)^{e_3} \qquad (6\text{-}7)$$

and (2) that the effect of pH is a constant, d_3, times a ratio of the concentration of algae to the critical concentration of algae which results in a death rate equal to d_3, (determined beforehand) then

$$d_3\ C_4 = d_3 \left(\frac{N_1}{N_{1C}} \right)^{e_4} \qquad (6\text{-}8)$$

The equation for the time-dependent changes in the Potamocypris population becomes

$$\frac{d\,N_2}{d\,t} = \left\{ b_2 \left[1 = \left(\frac{N_2}{N_{2M}} \right)^{e_3} \right] - d_2 - d_3 \left(\frac{N_1}{N_{1C}} \right)^{e_3} \right\} N_2 \qquad (6\text{-}9)$$

For constants that were unknown, values were substituted by trial and error until the combination of substituted values consistently simulated natural situations. Concentrations are expressed in mg/l and rates are expressed in mg/l day^{-1}. The final equations, with substituted constants, are:

$$\frac{d\,N_1}{d\,t} = (1) \left[1 - \left(\frac{N_1}{1000} \right)^4 \right] N_1 - .12 \left[1 - \left(\frac{50}{N_1} \right)^4 \right] N_2 \qquad (6\text{-}10)$$

$$\frac{d\,N_2}{d\,t} = \left\{ .2 \left[1 - \left(\frac{N_2}{7000} \right)^4 \right] - .01 - (1) \left(\frac{N_1}{600} \right)^2 \right\} N_2 \qquad (6\text{-}11)$$

A computer program, using the Runga Kutta integration routine, was used to find $N_1(t)$ and $N_2(t)$ for $0 \leq t \leq 5$ days. (See Appendix C) A variety of initial concentrations of algae and Potamocypris were tried with the object of proving the existence and, subsequently, determining the magnitude of the critical algae/Potamocypris ratio. In Figure 44 is indicated the initial conditions leading to victory for algae or ostracods. That is, initial concentrations above the line eventually result in a maximum concentration of ostracods and a minimum concentration of algae. Initial conditions below the line lead to the opposite result. The vertical asymptote of the curve results from the assumption that 600 mg/l of algae result in a pH above 10 and a highly toxic environment for ostracods as evidenced by the experimental data shown in Figure 39. For initial algae concentrations

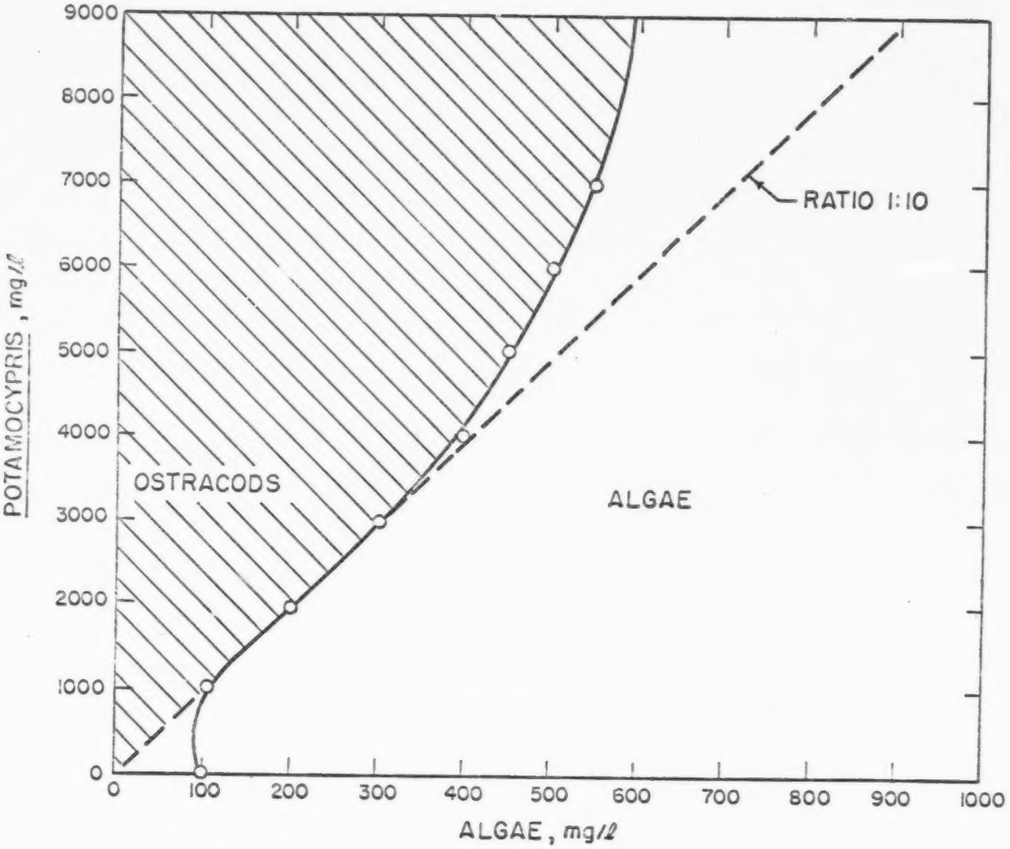

Figure 44. Initial Conditions Leading to Algae or Ostracod Predominance.

below 400 mg/1, the critical ratio seems to be approximately one to ten. That is, an initial condition of more than one mg/1 of algae for every ten of Potamocypris will lead to a maximum algal concentration and the exclusion of ostracods.

As an application of this information, assume a pond to be infested with 4000 mg/1 of Potamocypris. The critical ratio at this value is one to ten and therefore a concentration of algae greater than 400 mg/1 would have to be added at one time so that the initial concentration had a chance to raise the pH to a point where it would have an effect on the Potamocypris. In Figure 45 is shown this initial concentration of Potamocypris with various initial concentrations of algae. The curves show the changing (N_1, N_2) concentrations over a five-day period. As the initial combinations move away from the one to ten ratio, the curves are steeper, taking fewer days to reach either a maximum algae or Potamocypris.

DISCUSSION

It needs to be emphasized that Potamocypris is only one of the many possible predators in high-rate oxidation ponds and that pH is only one of its controlling factors. This study does not contradict the current hypothesis that a high BOD loading will prevent infestation. Instead, it suggests a possible plan to remove an already existing infestation by proposing that, given a Potamocypris tolerance level of a pH of ten, and given pH to a function of algae, a minimum innoculation of algae can be determined above which the algae should regain control of the pond.

The critical ratio of 1:10 was determined by making rational assumptions for the constants used in the model. Therefore, this ratio can't be used for solving real problems without further experimentation. Further study needs to be done in determining the constants used in the equations of the model and in detailed testing of this theory. Also, considerable work needs to be invested in finding other controlling factors causing predation in oxidation ponds. The knowledge gained in this area

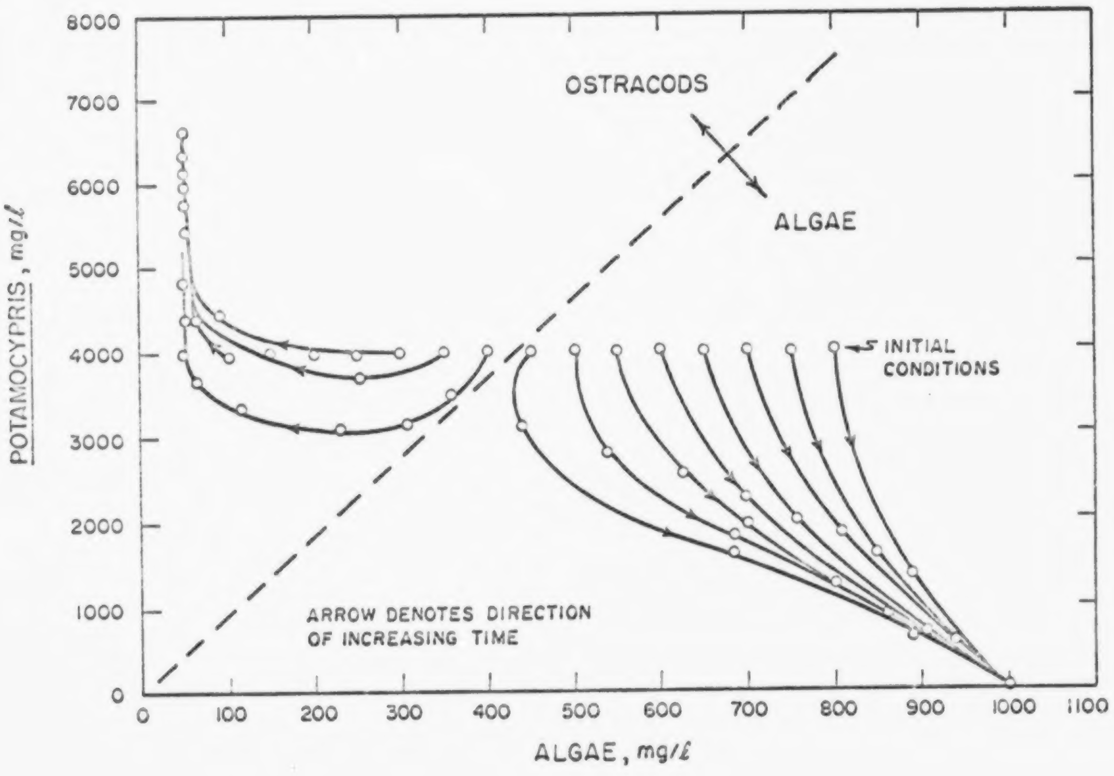

Figure 45. Population Concentrations as a Function of Time for Various Initial Conditions.

can help toward removing predation as a hindrance when large-scale production of algae becomes necessary.

SUMMARY

A study was made of predator-prey relationships in an oxidation pond in an attempt to find a biological solution to the problem of predation of the algae. An initial hypothesis, supported by the results of preliminary experiments, was made that the ostracod, Potamocypris, sp. would not survive in a well-working oxidation pond. Environmental conditions in an inoperative pond infested with Potamocypris were compared to four smaller, well-working oxidation ponds nearby. Diurnal studies were run on both types of ponds to determine changes in temperature, conductivity, pH, and dissolved oxygen. Of these, pH and dissolved oxygen differed sharply in the two types of ponds. Tests for tolerance levels in pH and dissolved oxygen were run with Potamocypris. The dissolved oxygen range of tolerance is wide and includes all levels encountered in both the inoperative and the well-working ponds. It was found in a test for pH tolerance that the viability of Potamocypris starts to fall off rapidly at a pH of 10. This was compared to the average diurnal pH of 10.3 in a well-working pond. In testing the validity of the pH tolerance range, it was found that there was little or no physiological adaptation to changes in pH and the effect of increased time at respective units of pH was of little significance except at a pH of 11.

A mathematical model was formulated to picture the dynamics of the predator-prey relationship in regards to this effect of pH. It was hypothesized that the population dynamics were dependent on the initial ratio of algal concentration to Potamocypris concentrations, and that if this concentration were above a certain value, the algae would increase to a maximum at the expense of the Potamocypris. Equations based on Gause's

predator-prey relationships were formulated, with constants assumed, and then analyzed using a digital computer for simulated five-day runs for various initial combinations of algae to Potamocypris. From these results it was found that the critical ratio of algae to ostracods hovered around 1:10. An example was presented to show how the application of this ratio could eliminate ostracods from an infested pond. The need for further study was emphasized.

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APPENDICES

A. GLOSSARY

B. COMPUTER PROGRAM FOR REGRESSION ANALYSIS

C. COMPUTER PROGRAM FOR MATHEMATICAL MODELS
OF PREDATOR-PREY RELATIONSHIP

APPENDIX A

GLOSSARY

BOD - Biochemical Oxygen Demand

c - Velocity of light $[LT^{-1}]$

C_c or X_1 - Algal concentration in suspension $[ML^{-3}]$

C_{co} - Algal concentration in the influent $[ML^{-3}]$

Ch - Chlorophyl content

D_R - Dilution Rate $[T^{-1}]$

d - Depth of algal reactor measured from the illuminated surface $[L]$

E - Quantum energy content; Energy units $[E]$

E_o - Overall light conversion efficiency of photosynthesis

E_e - Algal Conversion Efficiency on the basis of electric energy input

E_i - Exponential integral

Em - Thermodynamic efficiency of the photosynthetic energy conversion

Em' - Dimentional thermodynamic light conversion efficiency $[E^{-1}L^2]$

E_q - Quantum efficiency

E_s - Efficiency of light utilization within an illuminated algal suspension

E_T - Total light conversion efficiency

h - Plank's contsnt $[ET]$

h' - Culture height from the bottom rim of the Algatron drum $[L]$

I - Light intensity (irradiance) $[EL^{-2}T^{-1}]$

I_o - Incident light intensity on the illuminated surface (incident irradiance) $[EL^{-2}T^{-1}]$

$\bar{I}_o$ - Average incident light intensity on culture surface $[EL^{-2}T^{-1}]$

$I_{o\lambda}$ - Incident light intensity of a given wavelength band $[EL^{-2}T^{-1}]$

$I_{1/2}$ - Light intensity constant corresponds to $\mu_x = \frac{1}{2}\mu_x$ $[EL^{-2}T^{-1}]$

I_c - Apparent continuous light intensity due to the use of flashing light technique $[EL^{-2}T^{-1}]$

I_d - Light intensity at depth, d $[EL^{-2}T^{-1}]$

$I_{d\lambda}$ - Light intensity at depth d corresponding to a given wavelength band $[EL^{-2}T^{-1}]$

I_f - Flash light intensity $[EL^{-2}T^{-1}]$

I_s - Light saturation intensity $[EL^{-2}T^{-1}]$

I_x - Light intensity (irradiance) in an incremental depth (dx)

$I_{x\lambda}$ - Light intensity corresponding to a given wavelength band at incremental depth (dx) $[EL^{-2}T^{-1}]$

$I_{\lambda R}$ - Relative light energy correspond to a given wavelength band

J - Algae chemical energy content $[EM^{-1}]$

$\left.\begin{matrix} K, K_o, K_1, \\ k, k_1, k_n \end{matrix}\right\}$ - Constants

K_A - Substrate concentration constant as applied to the "Modified Exponential Function" $[ML^{-3}]$

K_d - Respiration and decay rate constant $[T^{-1}]$

K_s - Substrate concentration constant as applied to the Michaelis-Menton Equation $[ML^{-3}]$

$\left.\begin{matrix} k, k_D, k_s, k_s', \\ k_s'', k_r, k_r', \\ k_1, k_2 \end{matrix}\right\}$ - Various reaction velocity constants

L - Lenth units

M - Mass units

N_A - Avogadro's Number

N_c - Nitrogen cellular content

n - Number of quanta; any integer

P_a - Gross production of dry algal per unit of illuminated surface per unit of time $[ML^{-2}T^{-1}]$

P_a' - Surface gross production of algae when both light and nitrogen concentrations are limiting $[ML^{-2}T^{-1}]$

P_{a1}, P_{a2}, P_{a3} - Gross production of dry algae on the basis of macroscopic models I, II and III, respectively $[ML^{-2}T^{-1}]$

P_n - Total algal net production per unit of time $[MT^{-1}]$

P_n - Maximum algal net production $[MT^{-1}]$

P_n' - Net algal production in an optically dense culture in which nitrogen is limiting $[MT^{-1}]$

P_{na} - Net production of dry algae per unit of illuminated surface per unit of time $[ML^{-2}T^{-1}]$

P_{na_1}, P_{na_2}, P_{na_3} - Net production of dry algae on the basis of macroscopic models I, II and III, respectively $[ML^{-2}T^{-1}]$

$\hat{P}_{na}$ - Maximum algal net production per unit of illuminated area $[ML^{-2}T^{-1}]$

P_{nv} - Algal net production per unit of culture volume $[ML^{-3}T^{-1}]$

$\hat{P}_{nv}$ - Maximum algal net production per unit of culture volume $[ML^{-3}T^{-1}]$

Q - Flow rate $[L^3T^{-1}]$

Q_λ - Relative quantum yield coefficient, accounting for the algal action spectrum of an optically dense culture

q - Substrate cellular removal rate. Mass of nitrogen removed per unit of mass of algae present in the reactor per unit time $[T^{-1}]$

R_f - Flash ratio = $^{t}f/t_f + t_d$

R_t - The sum of the relative light energy in the visible range, $\sum_{=400}^{=700} I_{\lambda R}$

R_x - Rate of photosynthesis at incremental depth (dx) $[ML^{-2}T^{-1}]$

$\bar{r}$ - Average radius of the Algatrom drum $[L]$

S_N - Concentration of a nitrogenous compound expressed as N $[ML^{-3}]$

S_{N_1} - Substrate ammonia nitrogen concentration (effluent concentration and/or reactor concentration in CSTR-type process) $[ML^{-3}]$

S_{N_o} - Initial nitrogen concentration (influent concentration $[ML^{-3}]$

S_{NS} - Nitrogen concentration corresponds to $\mu_n = 0.9\ \hat{\mu}_n$

s' - Slope of culture surface in the Algatron

s_w - Slot width $[L]$

T - Time units

T_r - Percent of light transmission

t - Time

t' - Dummy variable of integration

t_d - Dark period $[T]$

t_f - Flash period $[T]$

U - Energy content of a mole of quanta (Einstein) $[E]$

V - Volume of growth unit $[L^3]$

X_1 or C_c - Algal concentration in suspension $[ML^{-3}]$

x - Length of light path within algal suspension $[L]$

x_s - Depth of culture corresponds to I_s $[L]$

Y - Yield coefficient of nitrogen removal. Mass of algae produced per a unit mass of nitrogen removed

$\bar{Y}$ - Average yield coefficient at the range of full growth

$\bar{Y}_{NS}$ - Yield coefficient as S_{NS}

α - Light extinction coefficient $[M^{-1}L^2]$

α_t - Integrated light extinction coefficient for polychromatic light $[M^{-1}L^2]$

α_λ - Light extinction coefficient for a beam of a particular wavelength $[M^{-1}L^2]$

γ - Fraction of light absorbed by a given algal suspension

γ' - Euler's constant

ΔH - Chemical energy content per mole $[E]$

θ - Mean residence time or average retention period $[T]$

θ_c - Mean cellular residence time $[T]$

λ - Wavelength $[L]$

$\bar{\mu}$ - Average specific growth rate $[T^{-1}]$

$\bar{\mu}_1, \bar{\mu}_2, \bar{\mu}_3$ - Average specific growth rates on the basis of macroscopic models I, II and III, respectively $[T^{-1}]$

μ_n - Net specific growth rate $[T^{-1}]$

$\bar{\mu}_n$ - Average net specific growth rate $[T^{-1}]$

$\hat{\mu}_n$ - Maximum net specific growth rate $[T^{-1}]$

μ_N' - Specific growth rate corresponding to a given growth-limiting nitrogen concentration $[T^{-1}]$

$\bar{\mu}_{n1}, \bar{\mu}_{n2}, \bar{\mu}_{n3}$ - Average net specific growth rate on the basis of macroscopic models I, II and III, respectively $[T^{-1}]$

$\bar{\mu}_{na}$ - Average net specific growth rate at an incremental surface area $[T^{-1}]$

μ_x - Specific growth rate in an incremental depth (dx) (specific growth rate on the microscopic level) $[T^{-1}]$

$\hat{\mu}_x$ - The microscopic maximum specific growth rate $[T^{-1}]$

ν - Wave frequency $[T^{-1}]$

Φ - Quantum yield; quantum efficiency

APPENDIX B

COMPUTER PROGRAM
FOR LINEAR REGRESSION AND CORRELATION OF A LOGARITHMIC FUNCTION

```
3400032007013600032007024902402511963611300102
ZZJOB 5
ZZFORX51
       NI=0
       AMU=0.
       SN=0.
       AMUT=4.32
       READ 30,N
    30 FORMAT (I4)
    40 READ 50,AMU,SN
    50 FORMAT (2F10.2)
       NI=NI+1
       Y=.4343*LOGF(AMUT)-.4343*LOGF(AMUT-AMU)
       X=SN
       PUNCH 100,X,Y .
   100 FORMAT (2F10.4)
       IF (N-NI) 150,150,40
   150 STOP 00150
   180 END
  LINEAR REGRESSION (IN TWO VARIABLES)  -  LEAST SOUARES FIT
  B J SOCKS            DECEMBER 30, 1961.
1 IF(SENSESWITCH1)2,3
2 ACCEPT101,N,M
  GO TO 4
3 READ101,N,M
4 EN=N
  EM=M
  SX=0.
  SY=0.
  SXY=0.
  SX2=0.
  SY2=0.
  DO5I=1,N
  READ102,X,Y
  SX=SX+X
  SY=SY+Y
  SXY=SXY+X*Y
  SX2=SX2+X*X
5 SY2=SY2+Y*Y
  XBAR=SX/EN
  YBAR=SY/EN
  CX2=SX*SX/EN
  CXY=SX*SY/EN
  VX2=SX2-CX2
  VXY=SXY-CXY
  B=VXY/VX2
  A=YBAR-B*XBAR
  C=SXY/SX2
```

```
      R=(((EN*SXY-SX*SY)**2)/((EN*SX2-SX*SX)*(EN*SY2-SY*SY)))**.5
      IF(R)6,7,7
    6 RA=-R
      GO TO 8
    7 RA=R
    8 S2SX=(EN*SX2-SX*SX)/(EN*(EN-1.))
      S2SY=(EN*SY2-SY*SY)/(EN*(EN-1.))
      S2YGX=((EN-1.)/(EN-2.))*(S2SY-B*B*S2SX)
      SYGX=S2YGX**.5
      SB=SYGX/((S2SX*(EN-1.))**.5)
      IF(B)9,10,10
    9 BA=-B
      GO TO 11
   10 BA=B
   11 T=BA/SB
      IF(T)12,13,13
   12 TA=-T
      GO TO 14
   13 TA=T
   14 DF2=EN-2.
      SEX=((SX2-SX*SX/EN)/(EN-1.))**.5
      SEY=((SY2-SY*SY/EN)/(EN-1.))**.5
      PRINT103
      PRINT104,N
      PRINT105
      PRINT106,A,B
      PRINT107
      PRINT108,C
      PRINT109



      PRINT110,XBAR
      PRINT117,SEX
      PRINT118,SEY
      PRINT111,RA
      PRINT112,SYGX
      PRINT113,SB
      PRINT114,TA
      PRINT115
      PRINT116,DF2
      IF(SENSESWITCH3)16,1
   16 SEXM=SEX*EM
      SEYM=SEY*EM
      SGM=SYGX*EM
      DO31I=1,N
      KW=0
      READ102,X,Y
      XD=XBAR-X
      YD=YBAR-Y
      YES=A+B*X
      SDO=Y-YES
```

APPENDIX B (cont.)

```
      IF(XD)17,18,18
   17 XD=-XD
   18 IF(YD)19,20,20
   19 YD=-YD
   20 IF(SDO)21,22,22
   21 SDO=-SDO
   22 IF(XD-SEXM)24,24,23
   23 KW=1
      GOTO28
   24 IF(YD-SEYM)26,26,25
   25 KW=1
      GOTO28
   26 IF(SDO-SGM)28,28,27
   27 KW=1
   28 IF(I-1)282,281,282
  281 PRINT119
      PRINT122
  282 IF(KW)29,29,30
   29 PRINT120,I,Y,YD,X,XD,YES,SDO
      GOTO31
   30 PRINT121,I,Y,YD,X,XD,YES,SDO
   31 CONTINUE
      PRINT123
      PRINT124
      GOTO1
  101 FORMAT(I4,I4)
  102 FORMAT(F10.3,F10.3)
  103 FORMAT(/21X,21H  LEAST  SQUARES  FIT)
  104 FORMAT(///5H FOR I4,14H  OBSERVATIONS)
  105 FORMAT(/47H FOR  THE  EQUATION  OF  THE  FORM  Y = A + B X)
  106 FORMAT(/26X,7H Y  =  F10.4,5H    +F10.4,3H  X)
  107 FORMAT(/43H FOR  THE  EQUATION  OF  THE  FORM  Y = C X)
  108 FORMAT(/26X,7H Y  =  F10.4,3H  X)
  109 FORMAT(///24H ADDITIONAL  INFORMATION)
  110 FORMAT(/35X,8H MEAN = F10.4)
  111 FORMAT(/16X,27H CORRELATION COEFFICIENT = F10.7)
  112 FORMAT(/13X,30H STANDARD ERROR OF ESTIMATE = F10.4)
  113 FORMAT(/43H      STANDARD ERROR OF COEFFICIENT,  B  = F10.7)
  114 FORMAT(/23X,20H THE STATISTIC  T = F10.5)
  115 FORMAT(///48H CORRELATION COEFFICIENT AND T ARE BOTH ABSOLUTE)
  116 FORMAT(/25H TABLE SHOULD BE CHECKED F6.0,19H DEGREES OF FREEDOM)



  117 FORMAT(/16X,27H STANDARD DEVIATION OF X = F10.5)
  118 FORMAT(/16X,27H STANDARD DEVIATION OF Y = F10.5)
  119 FORMAT(///49H ESTIMATION DATA FOR EQUATION RELIABILITY STUDIES)
  120 FORMAT(/I4,F10.4,F10.5,F10.4,F10.5,F10.4,F10.5)
  121 FORMAT(/I4,F10.4,F10.5,F10.4,F10.5,F10.4,F10.5,8H BAD OBS)
  122 FORMAT(///9X,1HY,8X,2HDY,9X,1HX,8X,2HDX,7X,5HY EST,4X,6HDY EST)
  123 FORMAT(///18H CASE IS COMPLETED)
  124 FORMAT(/46H TO PROCESS A NEW CASE, LOAD READER, HIT START)
      END
```

APPENDIX C

COMPUTER PROGRAM FOR MATHEMATICAL MODEL OF ALGAE-OSTRACOD RELATIONSHIP

```
PACE   1

// JCB

LCG CRIVE   CART SPEC   CART AVAIL  PHY DRIVE
  CCCC        4444        4444        0000

V2 MC7   ACTUAL  8K  CONFIG  8K

// FCR
*CNE KCRD INTEGERS
*ICCS(2501 READER,1403 PRINTER)
*LIST ALL
      EXTERNAL FCT,CUTP
      DIMENSICN PRMT(10),Y(10),DERY(10),AUX(8,10),BERY(10)
  10C FCRMAT(2I5,7F1C.1)
  101 FCRMAT(8F10.2)
  102 FORMAT(1H1,5X,'ALGAE-CSTRCCCD STUDY',//,5X,'J. HARRISON',//,10X,'D
     1AYS',9X,'ALGAE',8X,'CSTRCCCDS')
      READ(8,1C0)NDIM,NRUN,(PRMT(J),J=1,7)
      READ(8,1C1)(BERY(J),J=1,NDIM)
      DO 1 I=1,NRUN
      PRMT(6)=-1.
      WRITE(5,1C2)
      READ(8,101)(Y(J),J=1,NDIM)
      DC 2 J=1,NDIM
    2 DERY(J)=BERY(J)
      CALL RKGS(PRMT,Y,DERY,NDIM,IHLF,FCT,CUTP,AUX)
    1 CONTINUE
      END
```

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